(51) International Patent Classification ⁷ : C12Q 1/68	A2	(11) International Publication Number: WO 00/46405
		(43) International Publication Date: 10 August 2000 (10.08.00)

(21) International Application Number: PCT/US00/02882

(22) International Filing Date: 2 February 2000 (02.02.00)

(30) Priority Data:
09/243,022   2 February 1999 (02.02.99)   US

(71)(72) Applicant and Inventor: PALSSON, Bernhard [US/US]; 730 Fern Glen, La Jolla, CA 92037 (US).

(74) Agent: HUNT, Dale, C.; 16th Floor, 620 Newport Center Drive, Newport Beach, CA 92660 (US).

(81) Designated States: AE, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), DM, EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR (Utility model), KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: METHODS FOR IDENTIFYING DRUG TARGETS BASED ON GENOMIC SEQUENCE DATA

(57) Abstract

This invention provides a computational approach to identifying potential antibacterial drug targets based on a genome sequence and its annotation. Starting from a fully sequenced genome, open reading frame assignments are made which determine the metabolic genotype for the organism. The metabolic genotype, and more specifically its stoichiometric matrix, are analyzed using flux balance analysis to assess the effects of genetic deletions on the fitness of the organism and its ability to produce essential biomolecules required for growth.

START — 12

Obtain DNA Sequence of the Genome — 14

Determine Location of Open Reading Frames — 16

Assign Function to Genes Based on Homology of Open Reading Frames to Known Genes — 18

Determine Genes Involved in Cellular Metabolism — 20

END — 22

METHODS FOR IDENTIFYING DRUG TARGETS
BASED ON GENOMIC SEQUENCE DATA
Background of the Invention

Field of the Invention

5   This invention relates to methods for identifying drug targets based on genomic sequence data. More specifically, this invention relates to systems and methods for determining suitable molecular targets for the directed development of antimicrobial agents.

Description of the Related Art

Infectious disease is on a rapid rise and threatens to regain its status as a major health problem. Prior to the
10   discovery of antibiotics in the 1930s, infectious disease was a major cause of death. Further discoveries, development, and mass production of antibiotics throughout the 1940s and 1950s dramatically reduced deaths from microbial infections to a level where they effectively no longer represented a major threat in developed countries.

Over the years antibiotics have been liberally prescribed and the strong selection pressure that this represents has led to the emergence of antibiotic resistant strains of many serious human pathogens. In some cases
15   selected antibiotics, such as vancomycin, literally represent the last line of defense against certain pathogenic bacteria such as *Staphylococcus*. The possibility for staphylococci to acquire vancomycin resistance through exchange of genetic material with enterococci, which are commonly resistant to vancomycin, is a serious issue of concern to health care specialists. The pharmaceutical industry continues its search for new antimicrobial compounds, which is a lengthy and tedious, but very important process. The rate of development and introduction of new antibiotics appears to no
20   longer be able to keep up with the evolution of new antibiotic resistant organisms. The rapid emergence of antibiotic resistant organisms threatens to lead to a serious widespread health care concern.

The basis of antimicrobial chemotherapy is to selectively kill the microbe with minimal, and ideally no, harm to normal human cells and tissues. Therefore, ideal targets for antibacterial action are biochemical processes that are unique to bacteria, or those that are sufficiently different from the corresponding mammalian processes to allow
25   acceptable discrimination between the two. For effective antibiotic action it is clear that a vital target must exist in the bacterial cell and that the antibiotic be delivered to the target in an active form. Therefore resistance to an antibiotic can arise from: (i) chemical destruction or inactivation of the antibiotic; (ii) alteration of the target site to reduce or eliminate effective antibiotic binding; (iii) blocking antibiotic entry into the cell, or rapid removal from the cell after entry; and (iv) replacing the metabolic step inhibited by the antibiotic.

30   Thus, it is time to fundamentally re-examine the philosophy of microbial killing strategies and develop new paradigms. One such paradigm is a holistic view of cellular metabolism. The identification of "sensitive" metabolic steps in attaining the necessary metabolic flux distributions to support growth and survival that can be attacked to weaken or destroy a microbe, need not be localized to a single biochemical reaction or cellular process. Rather, different cellular targets that need not be intimately related in the metabolic topology could be chosen based on the
35   concerted effect the loss of each of these functions would have on metabolism.

A similar strategy with viral infections has recently proved successful. It has been shown that "cocktails" of different drugs that target different biochemical processes provide enhanced success in fighting against HIV infection. Such a paradigm shift is possible only if the necessary biological information as well as appropriate methods of rational analysis are available. Recent advances in the field of genomics and bioinformatics, in addition to mathematical

5   modeling, offer the possibility to realize this approach.

At present, the field of microbial genetics is entering a new era where the genomes of several microorganisms are being completely sequenced. It is expected that in a decade, or so, the nucleotide sequences of the genomes of all the major human pathogens will be completely determined. The sequencing of the genomes of pathogens such as *Haemophilus influenzae* has allowed researchers to compare the homology of proteins encoded by

10   the open reading frames (ORFs) with those of *Escherichia coli*, resulting in valuable insight into the *H. influenzae* metabolic features. Similar analyses, such as those performed with *H. influenzae*, will provide details of metabolism spanning the hierarchy of metabolic regulation from bacterial genomes to phenotypes.

These developments provide exciting new opportunities to carry out conceptual experiments *in silico* to analyze different aspects of microbial metabolism and its regulation. Further, the synthesis of whole-cell models is

15   made possible. Such models can account for each and every single metabolic reaction and thus enable the analysis of their role in overall cell function. To implement such analysis, however, a mathematical modeling and simulation framework is needed which can incorporate the extensive metabolic detail but still retain computational tractability. Fortunately, rigorous and tractable mathematical methods have been developed for the required systems analysis of metabolism.

20   A mathematical approach that is well suited to account for genomic detail and avoid reliance on kinetic complexity has been developed based on well-known stoichiometry of metabolic reactions. This approach is based on metabolic flux balancing in a metabolic steady state. The history of flux balance models for metabolic analyses is relatively short. It has been applied to metabolic networks, and the study of adipocyte metabolism. Acetate secretion from *E. coli* under ATP maximization conditions and ethanol secretion by yeast have also been investigated using this

25   approach.

The complete sequencing of a bacterial genome and ORF assignment provides the information needed to determine the relevant metabolic reactions that constitute metabolism in a particular organism. Thus a flux-balance model can be formulated and several metabolic analyses can be performed to extract metabolic characteristics for a particular organism. The flux balance approach can be easily applied to systematically simulate the effect of single, as

30   well as multiple, gene deletions. This analysis will provide a list of sensitive enzymes that could be potential antimicrobial targets.

The need to consider a new paradigm for dealing with the emerging problem of antibiotic resistant pathogens is a problem of vital importance. The route towards the design of new antimicrobial agents must proceed along directions that are different from those of the past. The rapid growth in bioinformatics has provided a wealth of

35   biochemical and genetic information that can be used to synthesize complete representations of cellular metabolism. These models can be analyzed with relative computational ease through flux-balance models and visual computing

techniques. The ability to analyze the global metabolic network and understand the robustness and sensitivity of its regulation under various growth conditions offers promise in developing novel methods of antimicrobial chemotherapy.

In one example, Pramanik et al. described a stoichiometric model of *E. coli* metabolism using flux-balance modeling techniques (*Stoichiometric Model of Escherichia coli Metabolism: Incorporation of Growth-Rate Dependent*
5   *Biomass Composition and Mechanistic Energy Requirements*, Biotechnology and Bioengineering, Vol. 56, No. 4, November 20, 1997). However, the analytical methods described by Pramanik, et al. can only be used for situations in which biochemical knowledge exists for the reactions occurring within an organism. Pramanik, et al. produced a metabolic model of metabolism for *E. coli* based on biochemical information rather than genomic data since the metabolic genes and related reactions for *E. coli* had already been well studied and characterized. Thus, this method is
10   inapplicable to determining a metabolic model for organisms for which little or no biochemical information on metabolic enzymes and genes is known. It can be envisioned that in the future the only information we may have regarding an emerging pathogen is its genomic sequence. What is needed in the art is a system and method for determining and analyzing the entire metabolic network of organisms whose metabolic reactions have not yet been determined from biochemical assays. The present invention provides such a system.

15   Summary of the Invention

This invention relates to constructing metabolic genotypes and genome specific stoichiometric matrices from genome annotation data. The functions of the metabolic genes in the target organism are determined by homology searches against databases of genes from similar organisms. Once a potential function is assigned to each metabolic gene of the target organism, the resulting data is analyzed. In one embodiment, each gene is subjected to a flux-
20   balance analysis to assess the effects of genetic deletions on the ability of the target organism to produce essential biomolecules necessary for its growth. Thus, the invention provides a high-throughput computational method to screen for genetic deletions which adversely affect the growth capabilities of fully sequenced organisms.

Embodiments of this invention also provide a computational, as opposed to an experimental, method for the rapid screening of genes and their gene products as potential drug targets to inhibit an organism's growth. This
25   invention utilizes the genome sequence, the annotation data, and the biomass requirements of an organism to construct genomically complete metabolic genotypes and genome-specific stoichiometric matrices. These stoichiometric matrices are analyzed using a flux-balance analysis. This invention describes how to assess the affects of genetic deletions on the fitness and productive capabilities of an organism under given environmental and genetic conditions.

30   Construction of a genome-specific stoichiometric matrix from genomic annotation data is illustrated along with applying flux-balance analysis to study the properties of the stoichiometric matrix, and hence the metabolic genotype of the organism. By limiting the constraints on various fluxes and altering the environmental inputs to the metabolic network, genetic deletions may be analyzed for their affects on growth. This invention is embodied in a software application that can be used to create the stoichiometric matrix for a fully sequenced and annotated genome.
35   Additionally, the software application can be used to further analyze and manipulate the network so as to predict the ability of an organism to produce biomolecules necessary for growth, thus, essentially simulating a genetic deletion.

Brief Description of the Drawings

Figure 1 is a flow diagram illustrating one procedure for creating metabolic genotypes from genomic sequence data for any organism.

Figure 2 is a flow diagram illustrating one procedure for producing in silico microbial strains from the metabolic genotypes created by the method of Figure 1, along with additional biochemical and microbiological data.

Figure 3 is a graph illustrating a prediction of genome scale shifts in transcription. The graph shows the different phases of the metabolic response to varying oxygen availability, starting from completely aerobic to completely anaerobic in *E. coli*. The predicted changes in expression pattern between phases II and V are indicated.

Detailed Description of the Preferred Embodiment

This invention relates to systems and methods for utilizing genome annotation data to construct a stoichiometric matrix representing most of all of the metabolic reactions that occur within an organism. Using these systems and methods, the properties of this matrix can be studied under conditions simulating genetic deletions in order to predict the affect of a particular gene on the fitness of the organism. Moreover, genes that are vital to the growth of an organism can be found by selectively removing various genes from the stoichiometric matrix and thereafter analyzing whether an organism with this genetic makeup could survive. Analysis of these lethal genetic mutations is useful for identifying potential genetic targets for anti-microbial drugs.

It should be noted that the systems and methods described herein can be implemented on any conventional host computer system, such as those based on Intel© microprocessors and running Microsoft Windows operating systems. Other systems, such as those using the UNIX or LINUX operating system and based on IBM®, DEC® or Motorola® microprocessors are also contemplated. The systems and methods described herein can also be implemented to run on client-server systems and wide-area networks, such as the Internet.

Software to implement the system can be written in any well-known computer language, such as Java, C, C + +, Visual Basic, FORTRAN or COBOL and compiled using any well-known compatible compiler.

The software of the invention normally runs from instructions stored in a memory on the host computer system. Such a memory can be a hard disk, Random Access Memory, Read Only Memory and Flash Memory. Other types of memories are also contemplated to function within the scope of the invention.

A process 10 for producing metabolic genotypes from an organism is shown in Figure 1. Beginning at a start state 12, the process 10 then moves to a state 14 to obtain the genomic DNA sequence of an organism. The nucleotide sequence of the genomic DNA can be rapidly determined for an organism with a genome size on the order of a few million base pairs. One method for obtaining the nucleotide sequences in a genome is through commercial gene databases. Many gene sequences are available on-line through a number of sites (see, for example, www.tigr.org) and can easily be downloaded from the Internet. Currently, there are 16 microbial genomes that have been fully sequenced and are publicly available, with countless others held in proprietary databases. It is expected that a number of other organisms, including pathogenic organisms will be found in nature for which little experimental information, except for its genome sequence, will be available.

Once the nucleotide sequence of the entire genomic DNA in the target organism has been obtained at state 14, the coding regions, also known as open reading frames, are determined at a state 16. Using existing computer algorithms, the location of open reading frames that encode genes from within the genome can be determined. For example, to identify the proper location, strand, and reading frame of an open reading frame one can perform a gene

5   search by signal (promoters, ribosomal binding sites, etc.) or by content (positional base frequencies, codon preference). Computer programs for determining open reading frames are available, for example, by the University of Wisconsin Genetics Computer Group and the National Center for Biotechnology Information.

After the location of the open reading frames have been determined at state 16, the process 10 moves to state 18 to assign a function to the protein encoded by the open reading frame. The discovery that an open reading

10   frame or gene has sequence homology to a gene coding for a protein of known function, or family of proteins of known function, can provide the first clues about the gene and it's related protein's function. After the locations of the open reading frames have been determined in the genomic DNA from the target organism, well-established algorithms (i.e. the Basic Local Alignment Search Tool (BLAST) and the FAST family of programs can be used to determine the extent of similarity between a given sequence and gene/protein sequences deposited in worldwide genetic databases. If a

15   coding region from a gene in the target organism is homologous to a gene within one of the sequence databases, the open reading frame is assigned a function similar to the homologously matched gene. Thus, the functions of nearly the entire gene complement or genotype of an organism can be determined so long as homologous genes have already been discovered.

All of the genes involved in metabolic reactions and functions in a cell comprise only a subset of the

20   genotype. This subset of genes is referred to as the metabolic genotype of a particular organism. Thus, the metabolic genotype of an organism includes most or all of the genes involved in the organism's metabolism. The gene products produced from the set of metabolic genes in the metabolic genotype carry out all or most of the enzymatic reactions and transport reactions known to occur within the target organism as determined from the genomic sequence.

To begin the selection of this subset of genes, one can simply search through the list of functional gene

25   assignments from state 18 to find genes involved in cellular metabolism. This would include genes involved in central metabolism, amino acid metabolism, nucleotide metabolism, fatty acid and lipid metabolism, carbohydrate assimilation, vitamin and cofactor biosynthesis, energy and redox generation, etc. This subset is generated at a state 20. The process 10 of determining metabolic genotype of the target organism from genomic data then terminates at an end stage 22.

30   Referring now to Figure 2, the process 50 of producing a computer model of an organism. This process is also known as producing *in silico* microbial strains. The process 50 begins at a start state 52 (same as end state 22 of process 10) and then moves to a state 54 wherein biochemical information is gathered for the reactions performed by each metabolic gene product for each of the genes in the metabolic genotype determined from process 10.

For each gene in the metabolic genotype, the substrates and products, as well as the stoichiometry of any

35   and all reactions performed by the gene product of each gene can be determined by reference to the biochemical

literature. This includes information regarding the irreverisble or reversible nature of the reactions. The stoichiometry of each reaction provides the molecular ratios in which reactants are converted into products.

Potentially, there may still remain a few reactions in cellular metabolism which are known to occur from *in vitro* assays and experimental data. These would include well characterized reactions for which a gene or protein has yet to be identified, or was unidentified from the genome sequencing and functional assignment of state 14 and 18. This would also include the transport of metabolites into or out of the cell by uncharacterized genes related to transport. Thus one reason for the missing gene information may be due to a lack of characterization of the actual gene that performs a known biochemical conversion. Therefore upon careful review of existing biochemical literature and available experimental data, additional metabolic reactions can be added to the list of metabolic reactions determined from the metabolic genotype from state 54 at a state 56. This would include information regarding the substrates, products, reversibilty/irreversibility, and stoichiometry of the reactions.

All of the information obtained at states 54 and 56 regarding reactions and their stoichiometry can be represented in a matrix format typically referred to as a stoichiometric matrix. Each column in the matrix corresponds to a given reaction or flux, and each row corresponds to the different metabolites involved in the given reaction/flux. Reversible reactions may either be represented as one reaction that operates in both the forward and reverse direction or be decomposed into one forward reaction and one backward reaction in which case all fluxes can only take on positive values. Thus, a given position in the matrix describes the stoichiometric participation of a metabolite (listed in the given row) in a particular flux of interest (listed in the given column). Together all of the columns of the genome specific stoichiometric matrix represent all of the chemical conversions and cellular transport processes that are determined to be present in the organism. This includes all internal fluxes and so called exchange fluxes operating within the metabolic network. Thus, the process 50 moves to a state 58 in order to formulate all of the cellular reactions together in a genome specific stoichiometric matrix. The resulting genome specific stoichiometric matrix is a fundamental representation of a genomically and biochemically defined genotype.

After the genome specific stoichiometric matrix is defined at state 58, the metabolic demands placed on the organism are calculated. The metabolic demands can be readily determined from the dry weight composition of the cell. In the case of well-studied organisms such as *Escherichia coli* and *Bacillus subtilis*, the dry weight composition is available in the published literature. However, in some cases it will be necessary to experimentally determine the dry weight composition of the cell for the organism in question. This can be accomplished with varying degrees of accuracy. The first attempt would measure the RNA, DNA, protein, and lipid fractions of the cell. A more detailed analysis would also provide the specific fraction of nucleotides, amino acids, etc. The process 50 moves to state 60 for the determination of the biomass composition of the target organism.

The process 50 then moves to state 62 to perform several experiments that determine the uptake rates and maintenance requirements for the organism. Microbiological experiments can be carried out to determine the uptake rates for many of the metabolites that are transported into the cell. The uptake rate is determined by measuring the depletion of the substrate from the growth media. The measurement of the biomass at each point is also required, in order to determine the uptake rate per unit biomass. The maintenance requirements can be determined from a

chemostat experiment. The glucose uptake rate is plotted versus the growth rate, and the y-intercept is interpreted as the non-growth associated maintenance requirements. The growth associated maintenance requirements are determined by fitting the model results to the experimentally determined points in the growth rate versus glucose uptake rate plot.

5   Next, the process 50 moves to a state 64 wherein information regarding the metabolic demands and uptake rates obtained at state 62 are combined with the genome specific stoichiometric matrix of step 8 together fully define the metabolic system using flux balance analysis (FBA). This is an approach well suited to account for genomic detail as it has been developed based on the well-known stoichiometry of metabolic reactions.

The time constants characterizing metabolic transients and/or metabolic reactions are typically very rapid, on
10   the order of milli-seconds to seconds, compared to the time constants of cell growth on the order of hours to days.Thus, the transient mass balances can be simplified to only consider the steady state behavior. Eliminating the time derivatives obtained from dynamic mass balances around every metabolite in the metabolic system, yields the system of linear equations represented in matrix notation,

$$S \bullet v - 0 \qquad \text{Equation 1}$$

15   where S refers to the stoichiometric matrix of the system, and v is the flux vector. This equation simply states that over long times, the formation fluxes of a metabolite must be balanced by the degradation fluxes. Otherwise, significant amounts of the metabolite will accumulate inside the metabolic network. Applying equation 1 to our system we let S now represent the genome specific stoichiometric matrix

To determine the metabolic capabilities of a defined metabolic genotype Equation 1 is solved for the
20   metabolic fluxes and the internal metabolic reactions, v, while imposing constraints on the activity of these fluxes. Typically the number of metabolic fluxes is greater than the number of mass balances (i.e., $m > n$) resulting in a plurality of feasible flux distributions that satisfy Equation 1 and any constraints placed on the fluxes of the system. This range of solutions is indicative of the flexibility in the flux distributions that can be achieved with a given set of metabolic reactions. The solutions to Equation 1 lie in a restricted region. This subspace defines the capabilities of
25   the *metabolic genotype* of a given organism, since the allowable solutions that satisfy Equation 1 and any constraints placed on the fluxes of the system define all the metabolic flux distributions that can be achieved with a particular set of metabolic genes.

The particular utilization of the metabolic genotype can be defined as the *metabolic phenotype* that is expressed under those particular conditions. Objectives for metabolic function can be chosen to explore the 'best' use
30   of the metabolic network within a given metabolic genotype. The solution to equation 1 can be formulated as a linear programming problem, in which the flux distribution that minimizes a particular objective if found. Mathematically, this optimization can be stated as;

$$\text{Minimize } Z \qquad \text{Equation 2}$$

35   $$\text{where } Z = \sum c_i \cdot v_i = \langle \mathbf{c} \bullet \mathbf{v} \rangle \qquad \text{Equation 3}$$

where Z is the objective which is represented as a linear combination of metabolic fluxes v_i. The optimization can also be stated as the equivalent maximization problem; i.e. by changing the sign on Z.

This general representation of Z enables the formulation of a number of diverse objectives. These objectives can be design objectives for a strain, exploitation of the metabolic capabilities of a genotype, or physiologically meaningful objective functions, such as maximum cellular growth. For this application, growth is to be defined in terms of biosynthetic requirements based on literature values of biomass composition or experimentally determined values such as those obtained from state 60. Thus, we can define biomass generation as an additional reaction flux draining intermediate metabolites in the appropriate ratios and represented as an objective function Z. In addition to draining intermediate metabolites this reaction flux can be formed to utilize energy molecules such as ATP, NADH and NADPH so as to incorporate any maintenance requirement that must be met. This new reaction flux then becomes another constraint/balance equation that the system must satisfy as the objective function. It is analogous to adding an addition column to the stoichiometric matrix of Equation 1 to represent such a flux to describe the production demands placed on the metabolic system. Setting this new flux as the objective function and asking the system to maximize the value of this flux for a given set of constraints on all the other fluxes is then a method to simulate the growth of the organism.

Using linear programming, additional constraints can be placed on the value of any of the fluxes in the metabolic network.

$$\beta_i \leq v_i \leq \alpha_i \qquad\qquad \text{Equation 4}$$

These constraints could be representative of a maximum allowable flux through a given reaction, possibly resulting from a limited amount of an enzyme present in which case the value for α_i would take on a finite value. These constraints could also be used to include the knowledge of the minimum flux through a certain metabolic reaction in which case the value for β_i would take on a finite value. Additionally, if one chooses to leave certain reversible reactions or transport fluxes to operate in a forward and reverse manner the flux may remain unconstrained by setting β_i to negative infinity and α_i to positive infinity. If reactions proceed only in the forward reaction β_i is set to zero while α_i is set to positive infinity. As an example, to simulate the event of a genetic deletion the flux through all of the corresponding metabolic reactions related to the gene in question are reduced to zero by setting β_i and α_i to be zero in Equation 4. Based on the *in vivo* environment where the bacteria lives one can determine the metabolic resources available to the cell for biosynthesis of essentially molecules for biomass. Allowing the corresponding transport fluxes to be active provides the *in silico* bacteria with inputs and ouputs for substrates and by-products produces by the metabolic network. Therefore as an example, if one wished to simulate the absence of a particular growth substrate one simply constrains the corresponding transport fluxes allowing the metabolite to enter the cell to be zero by allowing β_i and α_i to be zero in Equation 4. On the other hand if a substrate is only allowed to enter or exit the cell via transport mechanisms, the corresponding fluxes can be properly constrained to reflect this scenario.

Together the linear programming representation of the genome-specific stoichiometric matrix as in Equation 1 along with any general constraints placed on the fluxes in the system, and any of the possible objective functions

completes the formulation of the *in silico* bacterial strain. The *in silico* strain can then be used to study theoretical metabolic capabilities by simulating any number of conditions and generating flux distributions through the use of linear programming. The process 50 of formulating the *in silico* strain and simulating its behavior using linear programming techniques terminates at an end state 66.

5   Thus, by adding or removing constraints on various fluxes in the network it is possible to (1) simulate a genetic deletion event and (2) simulate or accurately provide the network with the metabolic resources present in its *in vivo* environment. Using flux balance analysis it is possible to determine the affects of the removal or addition of particular genes and their associated reactions to the composition of the metabolic genotype on the range of possible metabolic phenotypes. If the removal/deletion does not allow the metabolic network to produce necessary precursors

10   for growth, and the cell can not obtain these precursors from its environment, the deletion(s) has the potential as an antimicrobial drug target. Thus by adjusting the constraints and defining the objective function we can explore the capabilities of the metabolic genotype using linear programming to optimize the flux distribution through the metabolic network. This creates what we will refer to as an *in silico* bacterial strain capable of being studied and manipulated to analyze, interpret, and predict the genotype-phenotype relationship. It can be applied to assess the affects of

15   incremental changes in the genotype or changing environmental conditions, and provide a tool for computer aided experimental design. It should be realized that other types of organisms can similarly be represented *in silico* and still be within the scope of the invention.

The construction of a genome specific stoichiometric matrix and *in silico* microbial strains can also be applied to the area of signal transduction. The components of signaling networks can be identified within a genome and used

20   to construct a content matrix that can be further analyzed using various techniques to be determined in the future.

Example 1: E. coli metabolic genotype and in silico model

Using the methods disclosed in Figures 1 and 2, an *in silico* strain of *Escherichia coli* K-12 has been constructed and represents the first such strain of a bacteria largely generated from annotated sequence data and from biochemical information. The genetic sequence and open reading frame identifications and assignments are

25   readily available from a number of on-line locations (ex: www.tigr.org). For this example we obtained the annotated sequence from the following website for the *E. coli* Genome Project at the University of Wisconsin (http://www.genetics.wisc.edu/). Details regarding the actual sequencing and annotation of the sequence can be found at that site. From the genome annotation data the subset of genes involved in cellular metabolism was determined as described above in Figure 1, state 20, comprising the metabolic genotype of the particular strain of *E. coli*.

30   Through detailed analysis of the published biochemical literature on *E. coli* we determined (1) all of the reactions associated with the genes in the metabolic genotype and (2) any additional reactions known to occur from biochemical data which were not represented by the genes in the metabolic genotype. This provided all of the necessary information to construct the genome specific stoichiometric matrix for *E. coli* K-12.

Briefly, the *E. coli* K-12 bacterial metabolic genotype and more specifically the genome specific

35   stoichiometric matrix contains 731 metabolic processes that influence 436 metabolites (dimensions of the genome specific stoichiometric matrix are 436 x 731). There are 80 reactions present in the genome specific stoichiometric

matrix that do not have a genetic assignment in the annotated genome, but are known to be present from biochemical data. The genes contained within this metabolic genotype are shown in Table 1 along with the corresponding reactions they carry out.

Because *E. coli* is arguably the best studied organism, it was possible to determine the uptake rates and
5   maintenance requirements (state 62 of Figure 2) by reference to the published literature. This *in silico* strain accounts for the metabolic capabilities of *E. coli*. It includes membrane transport processes, the central catabolic pathways, utilization of alternative carbon sources and the biosynthetic pathways that generate all the components of the biomass. In the case of *E. coli* K-12, we can call upon the wealth of data on overall metabolic behavior and detailed biochemical information about the *in vivo* genotype to which we can compare the behavior of the *in silico* strain. One
10   utility of FBA is the ability to learn about the physiology of the particular organism and explore its metabolic capabilities without any specific biochemical data. This ability is important considering possible future scenarios in which the only data that we may have for a newly discovered bacterium (perhaps pathogenic) could be its genome sequence.

Example 2: in silico deletion analysis for E. coli to find antimicrobial targets

Using the *in silico* strain constructed in Example 1, the effect of individual deletions of all the enzymes in central metabolism can be examined *in silico*. For the analysis to determine sensitive linkages in the metabolic network of *E. coli*, the objective function utilized is the maximization of the biomass yield. This is defined as a flux draining the necessary biosynthetic precursors in the appropriate ratios. This flux is defined as the biomass composition, which can be determined from the literature. See Neidhardt et. al., *Escherichia coli and Salmonella*: Cellular and Molecular Biology, Second Edition, ASM Press, Washington D.C., 1996. Thus, the objective function is the maximization of a single flux, this biosynthetic flux.

Constraints are placed on the network to account for the availability of substrates for the growth of *E. coli*. In the initial deletion analysis, growth was simulated in an aerobic glucose minimal media culture. Therefore, the constraints are set to allow for the components included in the media to be taken up. The specific uptake rate can be included if the value is known, otherwise, an unlimited supply can be provided. The uptake rate of glucose and oxygen have been determined for *E. coli* (Neidhardt et. al., *Escherichia coli and Salmonella*: Cellular and Molecular Biology, Second Edition, ASM Press, Washington D.C., 1996. Therefore, these values are included in the analysis. The uptake rate for phosphate, sulfur, and nitrogen source is not precisely known, so constraints on the fluxes for the uptake of these important substrates is not included, and the metabolic network is allowed to take up any required amount of these substrates.

The results showed that a high degree of redundancy exists in central intermediary metabolism during growth in glucose minimal media, which is related to the interconnectivity of the metabolic reactions. Only a few metabolic functions were found to be essential such that their loss removes the capability of cellular growth on glucose. For growth on glucose, the essential gene products are involved in the 3-carbon stage of glycolysis, three reactions of the

TCA cycle, and several points within the PPP. Deletions in the 6-carbon stage of glycolysis result in a reduced ability to support growth due to the diversion of additional flux through the PPP.

The results from the gene deletion study can be directly compared with growth data from mutants. The growth characteristics of a series of *E. coli* mutants on several different carbon sources were examined (80 cases were determined from the literature), and compared to the *in silico* deletion results (Table 2). The majority (73 of 80 cases or 91%) of the mutant experimental observations are consistent with the predictions of the *in silico* study. The results from the *in silico* gene deletion analysis are thus consistent with experimental observations.

Example 3: Prediction of genome scale shifts in gene expression

Flux based analysis can be used to predict metabolic phenotypes under different growth conditions, such as substrate and oxygen availability. The relation between the flux value and the gene expression levels is non-linear, resulting in bifurcations and multiple steady states. However, FBA can give qualitative (on/off) information as well as
5   the relative importance of gene products under a given condition. Based on the magnitude of the metabolic fluxes, qualitative assessment of gene expression can be inferred.

Figure 3a shows the five phases of distinct metabolic behavior of *E. Coli* in response to varying oxygen availability, going from completely anaerobic (phase I) to completely aerobic (phase V). Figures 3b and 3c display lists of the genes that are predicted to be induced or repressed upon the shift from aerobic growth (phase V) to nearly
10 complete anaerobic growth (phase II). The numerical values shown in Figures 3b and 3c are the fold change in the magnitude of the fluxes calculated for each of the listed enzymes.

For this example, the objective of maximization of biomass yield is utilized (as described above). The constraints on the system are also set accordingly (as described above). However, in this example, a change in the availability of a key substrate is leading to changes in the metabolic behavior. The change in the parameter is reflected
15 as a change in the uptake flux. Therefore, the maximal allowable oxygen uptake rate is changed to generate this data. The figure demonstrates how several fluxes in the metabolic network will change as the oxygen uptake flux is continuously decreased. Therefore, the constraints on the fluxes is identical to what is described in the previous section, however, the oxygen uptake rate is set to coincide with the point in the diagram.

Corresponding experimental data sets are now becoming available. Using high-density oligonucleotide arrays
20 the expression levels of nearly every gene in *Saccharomyces cerevisiae* can now be analyzed under various growth conditions. From these studies it was shown that nearly 90% of all yeast mRNAs are present in growth on rich and minimal media, while a large number of mRNAs were shown to be differentially expressed under these two conditions. Another recent article shows how the metabolic and genetic control of gene expression can be studied on a genomic scale using DNA microarray technology (*Exploring the Metabolic and Genetic Control of Gene Expression on a Genomic*
25 *Scale,* Science, Vol. 278, October 24, 1997. The temporal changes in genetic expression profiles that occur during the diauxic shift in *S. cerevisiae* were observed for every known expressed sequence tag (EST) in this genome. As shown above, FBA can be used to qualitatively simulate shifts in metabolic genotype expression patterns due to alterations in

growth environments. Thus, FBA can serve to complement current studies in metabolic gene expression, by providing a fundamental approach to analyze, interpret, and predict the data from such experiments.

Example 4: Design of defined media

An important economic consideration in large-scale bioprocesses is optimal medium formulation. FBA can be
5   used to design such media. Following the approach defined above, a flux-balance model for the first completely sequenced free living organism, *Haemophilus influenzae*, has been generated. One application of this model is to predict a minimal defined media. It was found that *H. influenzae* can grow on the minimal defined medium as determined from the ORF assignments and predicted using FBA. Simulated bacterial growth was predicted using the following defined media: fructose, arginine, cysteine, glutamate, putrescine, spermidine, thiamin, NAD, tetrapyrrole,
10   pantothenate, ammonia, phosphate. This predicted minimal medium was compared to the previously published defined media and was found to differ in only one compound, inosine. It is known that inosine is not required for growth, however it does serve to enhance growth. Again the *in silico* results obtained were consistent with published *in vivo* research. These results provide confidence in the use of this type of approach for the design of defined media for organisms in which there currently does not exist a defined media.

15   While particular embodiments of the invention have been described in detail, it will be apparent to those skilled in the art that these embodiments are exemplary rather than limiting, and the true scope of the invention is defined by the claims that follow.

Table 1

The genes included in the *E. coli* metabolic genotype along with corresponding enzymes and reactions that comprise the genome specific stoichiometric matrix. The final column indicates the presence/absence of the gene (as the number of copies) in the *E. coli* genome. Thus the presence of a gene in the *E. coli* genome indicates that the gene is part of the metabolic genotype. Reactions/Genes not present in the genome are those gathered at state 56 in Figure 2 and together with the reactions of the genes in the metabolic genotype form the columns of the genome specific stoichiometric matrix.

Enzyme	Gene	Reaction	E. coli genome
Glucokinase	glk	GLC + ATP -> G6P + ADP	1
Glucokinase	glk	bDGLC + ATP -> 6DG6P + ADP	1
Phosphoglucose isomerase	pgi	G6P <-> F6P	1
Phosphoglucose isomerase	pgi	bDG6P <-> G6P	1
Phosphoglucose isomerase	pgi	bDG6P <-> F6P	1
Aldose 1-epimerase	galM	bDGLC <-> GLC	1
Glucose-1-phophatase	agp	G1P -> GLC + PI	1
Phosphofructokinase	pfkA	F6P + ATP -> FDP + ADP	1
Phosphofructokinase B	pfkB	F6P + ATP -> FDP + ADP	1
Fructose-1,6-bisphosphatase	fbp	FDP -> F6P + PI	1
Fructose-1,6-bisphosphate aldolase	fba	FDP <-> T3P1 + T3P2	2
Triosphosphate isomerase	tpiA	T3P1 <-> T3P2	1
Methylglyoxal synthase	mgsA	T3P2 -> MTHGXL + PI	0
Glyceraldehyde-3-phosphate dehydrogenase-A complex	gapA	T3P1 + PI + NAD <-> NADH + 13PDG	1
Glyceraldehyde-3-phosphate dehydrogenase-C complex	gapC1C2	T3P1 + PI + NAD <-> NADH + 13PDG	2

Enzyme	Gene	Reaction	E. coli genome
Phosphoglycerate kinase	pgk	13PDG + ADP <-> 3PG + ATP	1
Phosphoglycerate mutase 1	gpmA	3PG <-> 2PG	1
Phosphoglycerate mutase 2	gpmB	3PG <-> 2PG	1
Enolase	eno	2PG <-> PEP	1
Phosphoenolpyruvate synthase	ppsA	PYR + ATP -> PEP + AMP + PI	1
Pyruvate Kinase II	pykA	PEP + ADP -> PYR + ATP	1
Pyruvate Kinase I	pykF	PEP + ADP -> PYR + ATP	1
Pyruvate dehydrogenase	lpdA, aceEF	PYR + COA + NAD -> NADH + CO2 + ACCOA	3
Glucose-1-phosphate adenylyltransferase	glgC	ATP + G1P -> ADPGLC + PPI	1
Glycogen synthase	glgA	ADPGLC -> ADP + GLYCOGEN	1
Glycogen phosphorylase	glgP	GLYCOGEN + PI -> G1P	1
Maltodextrin phosphorylase	malP	GLYCOGEN + PI -> G1P	1
Glucose 6-phosphate-1-dehydrogenase	zwf	G6P + NADP <-> D6PGL + NADPH	1
6-Phosphogluconolactonase	pgl	D6PGL -> D6PGC	0
6-Phosphogluconate dehydrogenase (decarboxylating)	gnd	D6PGC + NADP -> NADPH + CO2 + RL5P	1
Ribose-5-phosphate isomerase A	rpiA	RL5P <-> R5P	1
Ribose-5-phosphate isomerase B	rpiB	RL5P <-> R5P	1
Ribulose phosphate 3-epimerase	rpe	RL5P <-> X5P	1
Transketolase I	tktA	R5P + X5P <-> T3P1 + S7P	1
Transketolase II	tktB	R5P + X5P <-> T3P1 + S7P	1
Transketolase I	tktA	X5P + E4P <-> F6P + T3P1	1

Enzyme	Gene	Reaction	E. coli genome
Transketolase II	tktB	X5P + E4P <-> F6P + T3P1	1
Transaldolase B	talB	T3P1 + S7P <-> E4P + F6P	1
Phosphogluconate dehydratase	edd	D6PGC -> 2KD6PG	1
2-Keto-3-deoxy-6-phosphogluconate aldolase	eda	2KD6PG -> T3P1 + PYR	1
Citrate synthase	gltA	ACCOA + OA -> COA + CIT	1
Aconitase A	acnA	CIT <-> ICIT	1
Aconitase B	acnB	CIT <-> ICIT	1
Isocitrate dehydrogenase	icdA	ICIT + NADP <-> CO2 + NADPH + AKG	1
2-Ketoglutarate dehydrogenase	sucAB, lpdA	AKG + NAD + COA -> CO2 + NADH + SUCCOA	3
Succinyl:CoA synthetase	sucCD	SUCCOA + ADP + PI <-> ATP + COA + SUCC	2
Succinate dehydrogenase	sdhABCD	SUCC + FAD -> FADH + FUM	4
Fumarate reductase	frdABCD	FUM + FADH -> SUCC + FAD	4
Fumarase A	fumA	FUM <-> MAL	1
Fumarase B	fumB	FUM <-> MAL	1
Fumarase C	fumC	FUM <-> MAL	1
Malate dehydrogenase	mdh	MAL + NAD <-> NADH + OA	1
D-Lactate dehydrogenase 1	dld	PYR + NADH <-> NAD + LAC	1
D-Lactate dehydrogenase 2	ldhA	PYR + NADH <-> NAD + LAC	1
Acetaldehyde dehydrogenase	adhE	ACCOA + 2 NADH <-> ETH + 2 NAD + COA	1
Pyruvate formate lyase 1	pflAB	PYR + COA -> ACCOA + FOR	2
Pyruvate formate lyase 2	pflCD	PYR + COA -> ACCOA + FOR	2

Enzyme	Gene	Reaction	E. coli genome
Formate hydrogen lyase	fdhF, hycBEFG	FOR -> CO2	5
Phosphotransacetylase	pta	ACCOA + PI <-> ACTP + COA	1
Acetate kinase A	ackA	ACTP + ADP <-> ATP + AC	1
GAR transformylase T	purT	ACTP + ADP <-> ATP + AC	1
Acetyl-CoA synthetase	acs	ATP + AC + COA -> AMP + PPI + ACCOA	1
Phosphoenolpyruvate carboxykinase	pckA	OA + ATP -> PEP + CO2 + ADP	1
Phosphoenolpyruvate carboxylase	ppc	PEP + CO2 -> OA + PI	1
Malic enzyme (NADP)	maeB	MAL + NADP -> CO2 + NADPH + PYR	0
Malic enzyme (NAD)	sfcA	MAL + NAD -> CO2 + NADH + PYR	1
Isocitrate lyase	aceA	ICIT -> GLX + SUCC	1
Malate synthase A	aceB	ACCOA + GLX -> COA + MAL	1
Malate synthase G	glcB	ACCOA + GLX -> COA + MAL	1
Inorganic pyrophosphatase	ppa	PPI -> 2 PI	1
NADH dehydrogenase II	ndh	NADH + Q -> NAD + QH2	1
NADH dehydrogenase I	nuoABEFGHIJKLMN	NADH + Q -> NAD + QH2 +3.5 HEXT	1
Formate dehydrogenase-N	fdnGHI	FOR + Q -> QH2 + CO2 +2 HEXT	3
Formate dehydrogenase-O	fdoHG	FOR + Q -> QH2 + CO2 +2 HEXT	3
Formate dehydrogenase	fdhF	FOR + Q -> QH2 + CO2 +2 HEXT	1
Pyruvate oxidase	poxB	PYR + Q -> AC + CO2 + QH2	1
Glycerol-3-phosphate dehydrogenase (aerobic)	glpD	GL3P + Q -> T3P2 + QH2	1
Glycerol-3-phosphate dehydrogenase (anaerobic)	glpABC	GL3P + Q -> T3P2 + QH2	3

Enzyme	Gene	Reaction	E. coli genome
Cytochrome oxidase bo3	cyoABCD, cycBC, cydAB	QH2 + .5 O2 -> Q + 2.5 HEXT	6
Cytochrome oxidase bd	cydABCD, appBC, cycBC	QH2 + .5 O2 -> Q + 2 HEXT	6
Succinate dehydrogenase complex	sdhABCD	FADH + Q <-> FAD + QH2	4
Thioredoxin reductase	trxB	OTHIO + NADPH -> NADP + RTHIO	1
Pyridine nucleotide transhydrogenase	pntAB	NADPH + NAD -> NADP + NADH	2
Pyridine nucleotide transhydrogenase	pntAB	NADP + NADH + 2 HEXT -> NADPH + NAD	2
Hydrogenase 1	hyaABC	2 Q + 2 HEXT <-> 2 QH2 + H2	3
Hydrogenase 2	hybAC	2 Q + 2 HEXT <-> 2 QH2 + H2	2
Hydrogenase 3	hycFGBE	2 Q + 2 HEXT <-> 2 QH2 + H2	4
F0F1-ATPase	atpABCDEFGHI	ATP <-> ADP + PI + 4 HEXT	9
Alpha-galactosidase (melibiase)	melA	MELI -> GLC + GLAC	1
Galactokinase	galK	GLAC + ATP -> GAL1P + ADP	1
Galactose-1-phosphate uridylyltransferase	galT	GAL1P + UDPG <-> G1P + UDPGAL	1
UDP-glucose 4-epimerase	galE	UDPGAL <-> UDPG	1
UDP-glucose-1-phosphate uridylyltransferase	galU	G1P + UTP <-> UDPG + PPI	1
Phosphoglucomutase	pgm	G1P <-> G6P	1
Periplasmic beta-glucosidase precursor	bglX	LCTS -> GLC + GLAC	1
Beta-galactosidase (LACTase)	lacZ	LCTS -> GLC + GLAC	1
trehalose-6-phosphate hydrolase	treC	TRE6P -> bDG6P + GLC	1
Beta-fructofuranosidase		SUC6P -> G6P + FRU	0
1-Phosphofructokinase (Fructose 1-phosphate kinase)	fruK	F1P + ATP -> FDP + ADP	1

Enzyme	Gene	Reaction	E. coli genome
Xylose isomerase	xylA	FRU -> GLC	1
Phosphomannomutase	cpsG	MAN6P <-> MAN1P	1
Mannose-6-phosphate isomerase	manA	MAN1P <-> F6P	1
N-Acetylglucosamine-6-phosphate deacetylase	nagA	NAGP -> GA6P + AC	1
Glucosamine-6-phosphate deaminase	nagB	GA6P -> F6P + NH3	1
N-Acetylneuraminate lyase	nanA	SLA -> PYR + NAMAN	1
L-Fucose isomerase	fucI	FUC <-> FCL	1
L-Fuculokinase	fucK	FCL + ATP -> FCL1P + ADP	1
L-Fuculose phosphate aldolase	fucA	FCL1P <-> LACAL + T3P2	1
Lactaldehyde reductase	fucO	LACAL + NADH <-> 12PPD + NAD	1
Aldehyde dehydrogenase A	aldA	LACAL + NAD <-> LLAC + NADH	1
Aldehyde dehydrogenase B	aldB	LACAL + NAD <-> LLAC + NADH	1
Aldehyde dehydrogenase	adhC	LACAL + NAD <-> LLAC + NADH	1
Aldehyde dehydrogenase	adhC	GLAL + NADH <-> GL + NAD	1
Aldehyde dehydrogenase	adhE	LACAL + NAD -> LLAC + NADH	1
Aldehyde dehydrogenase	aldH	LACAL + NAD <-> LLAC + NADH	1
Aldehyde dehydrogenase	aldH	ACAL + NAD -> AC + NADH	1
Gluconokinase I	gntV	GLCN + ATP -> D6PGC + ADP	1
Gluconokinase II	gntK	GLCN + ATP -> D6PGC + ADP	1
L-Rhamnose isomerase	rhaA	RMN <-> RML	1
Rhamnulokinase	rhaB	RML + ATP -> RML1P + ADP	1

Enzyme	Gene	Reaction	E. coli genome
Rhamnulose-1-phosphate aldolase	rhaD	RML1P <-> LACAL + T3P2	1
L-Arabinose isomerase	araA	ARAB <-> RBL	1
Arabinose-5-phosphate isomerase		RL5P <-> A5P	0
L-Ribulokinase	araB	RBL + ATP -> RL5P + ADP	1
L-Ribulose-phosphate 4-epimerase	araD	RL5P <-> X5P	1
Xylose isomerase	xylA	XYL <-> XUL	1
Xylulokinase	xylB	XUL + ATP -> X5P + ADP	1
Ribokinase	rbsK	RIB + ATP -> R5P + ADP	1
Mannitol-1-phosphate 5-dehydrogenase	mtlD	MNT6P + NAD <-> F6P + NADH	1
Glucitol-6-phosphate dehydrogenase	srlD	GLT6P + NAD <-> F6P + NADH	1
Galactitol-1-phosphate dehydrogenase	gatD	GLTL1P + NAD <-> TAG6P + NADH	1
Phosphofructokinase B	pfkB	TAG6P + ATP -> TAG16P + ADP	1
1-Phosphofructokinase	fruK	TAG6P + ATP -> TAG16P + ADP	1
Tagatose-6-phosphate kinase	agaZ	TAG6P + ATP -> TAG16P + ADP	1
Tagatose-bisphosphate aldolase 2	gatY	TAG16P <-> T3P2 + T3P1	1
Tagatose-bisphosphate aldolase 1	agaY	TAG16P <-> T3P2 + T3P1	1
Glycerol kinase	glpK	GL + ATP -> GL3P + ADP	1
Glycerol-3-phosphate-dehydrogenase-[NAD(P)+]	gpsA	GL3P + NADP <-> T3P2 + NADPH	1
Phosphopentomutase	deoB	DR1P <-> DR5P	1
Phosphopentomutase	deoB	R1P <-> R5P	1
Deoxyribose-phosphate aldolase	deoC	DR5P -> ACAL + T3P1	1

Enzyme	Gene	Reaction	E. coli genome
Asparate transaminase	aspC	OA + GLU <-> ASP + AKG	1
Asparagine synthetase (Glutamate dependent)	asnB	ASP + ATP + GLN -> GLU + ASN + AMP + PPI	1
Aspartate-ammonia ligase	asnA	ASP + ATP + NH3 -> ASN + AMP + PPI	1
Glutamate dehydrogenase	gdhA	AKG + NH3 + NADPH <-> GLU + NADP	1
Glutamate-ammonia ligase	glnA	GLU + NH3 + ATP -> GLN + ADP + PI	1
Glutamate synthase	gltBD	AKG + GLN + NADPH -> NADP + 2 GLU	2
Alanine transaminase	alaB	PYR + GLU <-> AKG + ALA	0
Valine-pyruvate aminotransferase	avtA	OIVAL + ALA -> PYR + VAL	1
Alanine racemase, biosynthetic	alr	ALA <-> DALA	1
Alanine racemase, catabolic	dadX	ALA -> DALA	1
N-Acetylglutamate synthase	argA	GLU + ACCOA -> COA + NAGLU	1
N-Acetylglutamate kinase	argB	NAGLU + ATP -> ADP + NAGLUYP	1
N-Acetylglutamate phosphate reductase	argC	NAGLUYP + NADPH <-> NADP + PI + NAGLUSAL	1
Acetylornithine transaminase	argD	NAGLUSAL + GLU <-> AKG + NAARON	1
Acetylornithine deacetylase	argE	NAARON -> AC + ORN	1
Carbamoyl phosphate synthetase	carAB	GLN + 2 ATP + CO2 -> GLU + CAP + 2 ADP + PI	2
Ornithine carbamoyl transferase 1	argF	ORN + CAP <-> CITR + PI	2
Ornithine carbamoyl transferase 2	argI	ORN + CAP <-> CITR + PI	1
Ornithine transaminase	ygjGH	ORN + AKG -> GLUGSAL + GLU	2
Argininosuccinate synthase	argG	CITR + ASP + ATP -> AMP + PPI + ARGSUCC	1
Argininosuccinate lyase	argH	ARGSUCC <-> FUM + ARG	1

Enzyme	Gene	Reaction	E. coli genome
Arginine decarboxylase, biosynthetic	speA	ARG -> CO2 + AGM	1
Arginine decarboxylase, degradative	adi	ARG -> CO2 + AGM	1
Agmatinase	speB	AGM -> UREA + PTRC	1
Ornithine decarboxylase, biosynthetic	speC	ORN -> PTRC + CO2	1
Ornithine decarboxylase, degradative	speF	ORN -> PTRC + CO2	1
Adenosylmethionine decarboxylase	speD	SAM <-> DSAM + CO2	1
Spermidine synthase	speE	PTRC + DSAM -> SPMD + 5MTA	1
Methylthioadenosine nucleosidase		5MTA -> AD + 5MTR	0
5-Methylthioribose kinase		5MTR + ATP -> 5MTRP + ADP	0
5-Methylthioribose-1-phosphate isomerase		5MTRP <-> 5MTR1P	0
E-1 (Enolase-phosphatase)		5MTR1P -> DKMPP	0
E-3 (Unknown)		DKMPP -> FOR + KMB	0
Transamination (Unknown)		KMB + GLN -> GLU + MET	0
γ-Glutamyl kinase	proB	GLU + ATP -> ADP + GLUP	1
Glutamate-5-semialdehyde dehydrogenase	proA	GLUP + NADPH -> NADP + PI + GLUGSAL	1
N-Acetylornithine deacetylase	argE	NAGLUSAL -> GLUGSAL + AC	1
Pyrroline-5-carboxylate reductase	proC	GLUGSAL + NADPH -> PRO + NADP	1
Threonine dehydratase, biosynthetic	ilvA	THR -> NH3 + OBUT	1
Threonine dehydratase, catabolic	tdcB	THR -> NH3 + OBUT	1
Acetohydroxybutanoate synthase I	ilvBN	OBUT + PYR -> ABUT + CO2	2
Acetohydroxybutanoate synthase II	ilvG1 2)M	OBUT + PYR -> ABUT + CO2	3

Enzyme	Gene	Reaction	E. coli genome
Acetohydroxybutanoate synthase III	ilvH	OBUT + PYR -> ABUT + CO2	2
Acetohydroxy Acid isomeroreductase	ilvC	ABUT + NADPH -> NADP + DHMVA	1
Dihydroxy acid dehydratase	ilvD	DHMVA -> OMVAL	1
Branched chain amino acid aminotransferase	ilvE	OMVAL + GLU <-> AKG + ILE	1
Acetolactate synthase I	ilvBN	2 PYR -> CO2 + ACLAC	2
Acetolactate synthase II	ilvG(12)M	2 PYR -> CO2 + ACLAC	3
Acetolactate synthase III	ilvIH	2 PYR -> CO2 + ACLAC	2
Acetohydroxy acid isomeroreductase	ilvC	ACLAC + NADPH -> NADP + DHVAL	1
Dihydroxy acid dehydratase	ilvD	DHVAL -> OIVAL	1
Branched chain amino acid aminotransferase	ilvE	OIVAL + GLU -> AKG + VAL	1
Valine-pyruvate aminotransferase	avtA	OIVAL + ALA -> PYR + VAL	1
Isopropylmalate synthase	leuA	ACCOA + OIVAL -> COA + CBHCAP	1
Isopropylmalate isomerase	leuCD	CBHCAP <-> IPPMAL	2
3-Isopropylmalate dehydrogenase	leuB	IPPMAL + NAD -> NADH + OICAP + CO2	1
Branched chain amino acid aminotransferase	ilvE	OICAP + GLU -> AKG + LEU	1
Aromatic amino acid transaminase	tyrB	OICAP + GLU -> AKG + LEU	1
2-Dehydro-3-deoxyphosphoheptonate aldolase F	aroF	E4P + PEP -> PI + 3DDAH7P	1
2-Dehydro-3-deoxyphosphoheptonate aldolase G	aroG	E4P + PEP -> PI + 3DDAH7P	1
2-Dehydro-3-deoxyphosphoheptonate aldolase H	aroH	E4P + PEP -> PI + 3DDAH7P	1
3-Dehydroquinate synthase	aroB	3DDAH7P -> DQT + PI	1
3-Dehydroquinate dehydratase	aroD	DQT <-> DHSK	1

Enzyme	Gene	Reaction	E. coli genome
Shikimate dehydrogenase	aroE	DHSK + NADPH <-> SME + NADP	1
Shikimate kinase I	aroK	SME + ATP -> ADP + SME5P	1
Shikimate kinase II	aroL	SME + ATP -> ADP + SME5P	1
3-Phosphoshikimate-1-carboxyvinyltransferase	aroA	SME5P + PEP <-> 3PSME + PI	1
Chorismate synthase	aroC	3PSME -> PI + CHOR	1
Chorismate mutase 1	pheA	CHOR -> PHEN	1
Prephenate dehydratase	pheA	PHEN -> CO2 + PHPYR	1
Aromatic amino acid transaminase	tyrB	PHPYR + GLU <-> AKG + PHE	1
Chorismate mutase 2	tyrA	CHOR -> PHEN	1
Prephanate dehydrogenase	tyrA	PHEN + NAD -> HPHPYR + CO2 + NADH	1
Aromatic amino acid transaminase	tyrB	HPHPYR + GLU <-> AKG + TYR	1
Asparate transaminase	aspC	HPHPYR + GLU <-> AKG + TYR	1
Anthranilate synthase	trpDE	CHOR + GLN -> GLU + PYR + AN	2
Anthranilate synthase component II	trpD	AN + PRPP -> PPI + NPRAN	1
Phosphoribosyl anthranilate isomerase	trpC	NPRAN -> CPAD5P	1
Indoleglycerol phosphate synthase	trpC	CPAD5P -> CO2 + IGP	1
Tryptophan synthase	trpAB	IGP + SER -> T3P1 + TRP	2
Phosphoribosyl pyrophosphate synthase	prsA	R5P + ATP <-> PRPP + AMP	1
ATP phosphoribosyltransferase	hisG	PRPP + ATP -> PPI + PRBATP	1
Phosphoribosyl-ATP pyrophosphatase	hisIE	PRBATP -> PPI + PRBAMP	1
Phosphoribosyl-AMP cyclohydrolase	hisIE	PRBAMP -> PRFP	1

-23-

Enzyme	Gene	Reaction	E. coli genome
Phosphoribosylformimino-5-amino-1-phosphoribosyl-4-imidazole carboxamide isomerase	hisA	PRFP -> PRLP	1
Imidazoleglycerol phosphate synthase	hisFH	PRLP + GLN -> GLU + AICAR + DIMGP	2
Imidazoleglycerol phosphate dehydratase	hisB	DIMGP -> IMACP	1
L-Histidinol phosphate aminotransferase	hisC	IMACP + GLU -> AKG + HISOLP	1
Histidinol phosphatase	hisB	HISOLP -> PI + HISOL	1
Histidinol dehydrogenase	hisD	HISOL + 3 NAD -> HIS + 3 NADH	1
3-Phosphoglycerate dehydrogenase	serA	3PG + NAD -> NADH + PHP	1
Phosphoserine transaminase	serC	PHP + GLU -> AKG + 3PSER	1
Phosphoserine phosphatase	serB	3PSER -> PI + SER	1
Glycine hydroxymethyltransferase	glyA	THF + SER -> GLY + METTHF	1
Threonine dehydrogenase	tdh	THR + COA -> GLY + ACCOA	1
Amino ketobutyrate CoA ligase	kbl	THR + COA -> GLY + ACCOA	1
Sulfate adenylyltransferase	cysDN	SLF + ATP + GTP -> PPI + APS + GDP + PI	2
Adenylylsulfate kinase	cysC	APS + ATP -> ADP + PAPS	1
3'-Phospho-adenylylsulfate reductase	cysH	PAPS + RTHIO -> OTHIO + H2SO3 + PAP	1
Sulfite reductase	cysIJ	H2SO3 + 3 NADPH <-> H2S + 3 NADP	2
Serine transacetylase	cysE	SER + ACCOA <-> COA + ASER	1
O-Acetylserine (thiol)-lyase A	cysK	ASER + H2S -> AC + CYS	1
O-Acetylserine (thiol)-lyase B	cysM	ASER + H2S -> AC + CYS	1
3' - 5' Bisphosphate nucleotidase		PAP -> AMP + PI	0

Enzyme	Gene	Reaction	E. coli genome
Aspartate kinase I	thrA	ASP + ATP <-> ADP + BASP	1
Aspartate kinase II	metL	ASP + ATP <-> ADP + BASP	1
Aspartate kinase III	lysC	ASP + ATP <-> ADP + BASP	1
Aspartate semialdehyde dehydrogenase	asd	BASP + NADPH <-> NADP + PI + ASPSA	1
Homoserine dehydrogenase I	thrA	ASPSA + NADPH <-> NADP + HSER	1
Homoserine dehydrogenase II	metL	ASPSA + NADPH <-> NADP + HSER	1
Homoserine kinase	thrB	HSER + ATP -> ADP + PHSER	1
Threonine synthase	thrC	PHSER -> PI + THR	1
Dihydrodipicolinate synthase	dapA	ASPSA + PYR -> D23PIC	1
Dihydrodipicolinate reductase	dapB	D23PIC + NADPH -> NADP + PIP26DX	1
Tetrahydrodipicolinate succinylase	dapD	PIP26DX + SUCCOA -> COA + NS2A6O	1
Succinyl diaminopimelate aminotransferase	dapC	NS2A6O + GLU <-> AKG + NS26DP	0
Succinyl diaminopimelate desuccinylase	dapE	NS26DP -> SUCC + D26PIM	1
Diaminopimelate epinerase	dapF	D26PIM <-> MDAP	1
Diaminopimelate decarboxylase	lysA	MDAP -> CO2 + LYS	1
Lysine decarboxylase 1	cadA	LYS -> CO2 + CADV	1
Lysine decarboxylase 2	ldcC	LYS -> CO2 + CADV	1
Homoserine transsuccinylase	metA	HSER + SUCCOA -> COA + OSLHSER	1
O-succinlyhomoserine lyase	metB	OSLHSER + CYS -> SUCC + LLCT	1
Cystathionine-β-lyase	metC	LLCT -> HCYS + PYR + NH3	1
Adenosyl homocysteinase (Unknown)	Unknown	HCYS + ADN <-> SAH	0

Enzyme	Gene	Reaction	E. coli genome
Cobalamin-dependent methionine synthase	metH	HCYS + MTHF -> MET + THF	1
Cobalamin-independent methionine synthase	metE	HCYS + MTHF -> MET + THF	1
S-Adenosylmethionine synthetase	metK	MET + ATP -> PPI + PI + SAM	1
D-Amino acid dehydrogenase	dadA	DALA + FAD -> FADH + PYR + NH3	1
Putrescine transaminase	pat	PTRC + AKG -> GABAL + GLU	0
Amino oxidase	tynA	PTRC -> GABAL + NH3	1
Aminobutyraldehyde dehydrogenase	prr	GABAL + NAD -> GABA + NADH	0
Aldehyde dehydrogenase	aldH	GABAL + NAD -> GABA + NADH	1
Aminobutyrate aminotransaminase 1	gabT	GABA + AKG -> SUCCSAL + GLU	1
Aminobutyrate aminotransaminase 2	goaG	GABA + AKG -> SUCCSAL + GLU	1
Succinate semialdehyde dehydrogenase –NAD	sad	SUCCSAL + NAD -> SUCC + NADH	0
Succinate semialdehyde dehydrogenase –NADP	gabD	SUCCSAL + NADP -> SUCC + NADPH	1
Asparininase I	ansA	ASN -> ASP + NH3	1
Asparininase II	ansB	ASN -> ASP + NH3	1
Aspartate ammonia-lyase	aspA	ASP -> FUM + NH3	1
Tryptophanase	tnaA	CYS -> PYR + NH3 + H2S	1
L-Cysteine desulfhydrase		CYS -> PYR + NH3 + H2S	0
Glutamate decarboxylase A	gadA	GLU -> GABA + CO2	1
Glutamate decarboxylase B	gadB	GLU -> GABA + CO2	1
Glutaminase A		GLN -> GLU + NH3	0
Glutaminase B		GLN -> GLU + NH3	0

Enzyme	Gene	Reaction	E. coli genome
Proline dehydrogenase	putA	PRO + FAD -> FADH + GLUGSAL	1
Pyrroline-5-carboxylate dehydrogenase	putA	GLUGSAL + NAD -> NADH + GLU	1
Serine deaminase 1	sdaA	SER -> PYR + NH3	1
Serine deaminase 2	sdaB	SER -> PYR + NH3	1
Tryptothanase	tnaA	SER -> PYR + NH3	1
D-Serine deaminase	dsdA	DSER -> PYR + NH3	1
Threonine dehydrogenase	tdh	THR + NAD -> 2A3O + NADH	1
Amino ketobutyrate ligase	kbl	2A3O + COA -> ACCOA + GLY	1
Threonine dehydratase catabolic	tdcB	THR -> OBUT + NH3	1
Threonine deaminase 1	sdaA	THR -> OBUT + NH3	1
Threonine deaminase 2	sdaB	THR -> OBUT + NH3	1
Tryptophanase	tnaA	TRP <-> INDOLE + PYR + NH3	1
Amidophosphoribosyl transferase	purF	PRPP + GLN -> PPI + GLU + PRAM	1
Phosphoribosylamine-glycine ligase	purD	PRAM + ATP + GLY <-> ADP + PI + GAR	1
Phosphoribosylglycinamide formyltransferase	purN	GAR + FTHF -> THF + FGAR	1
GAR transformylase T	purT	GAR + FOR + ATP -> ADP + PI + FGAR	1
Phosphoribosylformylglycinamide synthetase	purL	FGAR + ATP + GLN -> GLU + ADP + PI + FGAM	1
Phosphoribosylformylglycinamide cyclo-ligase	purM	FGAM + ATP -> ADP + PI + AIR	1
Phosphoribosylaminoimidazole carboxylase 1	purK	AIR + CO2 + ATP <-> NCAIR + ADP + PI	1
Phosphoribosylaminoimidazole carboxylase 2	purE	NCAIR <-> CAIR	1
Phosphoribosylaminoimidazole-succinocarboxamide synthetase	purC	CAIR + ATP + ASP <-> ADP + PI + SAICAR	1

Enzyme	Gene	Reaction	E. coli genome
5'-Phosphoribosyl-4-(N-succinocarboxamide)-5-aminoimidazole lyase	purB	SAICAR <-> FUM + AICAR	1
AICAR transformylase	purH	AICAR + FTHF <-> THF + PRFICA	1
IMP cyclohydrolase	purH	PRFICA <-> IMP	1
Adenylosuccinate synthetase	purA	IMP + GTP + ASP -> GDP + PI + ASUC	1
Adenylosuccinate lyase	purB	ASUC <-> FUM + AMP	1
IMP dehydrogenase	guaB	IMP + NAD -> NADH + XMP	1
GMP synthase	guaA	XMP + ATP + GLN -> GLU + AMP + PPI + GMP	1
GMP reductase	guaC	GMP + NADPH -> NADP + IMP + NH3	1
Aspartate-carbamoyltransferase	pyrBI	CAP + ASP -> CAASP + PI	2
Dihydroorotase	pyrC	CAASP <-> DOROA	1
Dihydroorotate dehydrogenase	pyrD	DOROA + Q <-> QH2 + OROA	1
Orotate phosphoribosyl transferase	pyrE	OROA + PRPP <-> PPI + OMP	1
OMP decarboxylase	pyrF	OMP -> CO2 + UMP	1
CTP synthetase	pyrG	UTP + GLN + ATP -> GLU + CTP + ADP + PI	1
Adenylate kinase	adk	ATP + AMP <-> 2 ADP	1
Adenylate kinase	adk	GTP + AMP <-> ADP + GDP	1
Adenylate kinase	adk	ITP + AMP <-> ADP + IDP	1
Adenylate kinase	adk	DAMP + ATP <-> ADP + DADP	1
Guanylate kinase	gmk	GMP + ATP <-> GDP + ADP	1
Deoxyguanylate kinase	gmk	DGMP + ATP <-> DGDP + ADP	1

Enzyme	Gene	Reaction	E. coli genome
Nucleoside-diphosphate kinase	ndk	GDP + ATP <-> GTP + ADP	1
Nucleoside-diphosphate kinase	ndk	UDP + ATP <-> UTP + ADP	1
Nucleoside-diphosphate kinase	ndk	CDP + ATP <-> CTP + ADP	1
Nucleoside-diphosphate kinase	ndk	DGDP + ATP <-> DGTP + ADP	1
Nucleoside-diphosphate kinase	ndk	DUDP + ATP <-> DUTP + ADP	1
Nucleoside-diphosphate kinase	ndk	DCDP + ATP <-> DCTP + ADP	1
Nucleoside-diphosphate kinase	ndk	DADP + ATP <-> DATP + ADP	1
Nucleoside-diphosphate kinase	ndk	DTDP + ATP <-> DTTP + ADP	1
AMP Nucleosidse	amn	AMP -> AD + R5P	1
Adenosine deaminase	add	ADN -> INS + NH3	1
Deoxyadenosine deaminase	add	DA -> DIN + NH3	1
Adenine deaminase	yicP	AD -> NH3 + HYXN	1
Inosine kinase	gsk	INS + ATP -> IMP + ADP	1
Guanosine kinase	gsk	GSN + ATP -> GMP + ADP	1
Adenosine kinase	adk	ADN + ATP -> AMP + ADP	1
Adenine phosphoryltransferase	apt	AD + PRPP -> PPI + AMP	1
Xanthine-guanine phosphoribosyltransferase	gpt	XAN + PRPP -> XMP + PPI	1
Xanthine-guanine phosphoribosyltransferase	gpt	HYXN + PRPP -> PPI + IMP	1
Hypoxanthine phosphoribosyltransferase	hpt	HYXN + PRPP -> PPI + IMP	1
Xanthine-guanine phosphoribosyltransferase	gpt	GN + PRPP -> PPI + GMP	1
Hypoxanthine phosphoribosyltransferase	hpt	GN + PRPP -> PPI + GMP	1

Enzyme	Gene	Reaction	E. coli genome
Xanthosine phosphorylase	xapA	DIN + PI <-> HYXN + DR1P	1
Purine nucleotide phosphorylase	deoD	DIN + PI <-> HYXN + DR1P	1
Xanthosine phosphorylase	xapA	DA + PI <-> AD + DR1P	1
Purine nucleotide phosphorylase	deoD	DA + PI <-> AD + DR1P	1
Xanthosine phosphorylase	xapA	DG + PI <-> GN + DR1P	1
Purine nucleotide phosphorylase	deoD	DG + PI <-> GN + DR1P	1
Xanthosine phosphorylase	xapA	HYXN + R1P <-> INS + PI	1
Purine nucleotide phosphorylase	deoD	HYXN + R1P <-> INS + PI	1
Xanthosine phosphorylase	xapA	AD + R1P <-> PI + ADN	1
Purine nucleotide phosphorylase	deoD	AD + R1P <-> PI + ADN	1
Xanthosine phosphorylase	xapA	GN + R1P <-> PI + GSN	1
Purine nucleotide phosphorylase	deoD	GN + R1P <-> PI + GSN	1
Xanthosine phosphorylase	xapA	XAN + R1P <-> PI + XTSN	1
Purine nucleotide phosphorylase	deoD	XAN + R1P <-> PI + XTSN	1
Uridine phosphorylase	udp	URI + PI <-> URA + R1P	1
Thymidine (deoxyuridine) phosphorylase	deoA	DU + PI <-> URA + DR1P	1
Purine nucleotide phosphorylase	deoD	DU + PI <-> URA + DR1P	1
Thymidine (deoxyuridine) phosphorylase	deoA	DT + PI <-> THY + DR1P	1
Cytidylate kinase	cmkA	DCMP + ATP <-> ADP + DCDP	1
Cytidylate kinase	cmkA	CMP + ATP <-> ADP + CDP	1
Cytidylate kinase	cmkB	DCMP + ATP <-> ADP + DCDP	1

Enzyme	Gene	Reaction	E. coli genome
Cytidylate kinase	cmkB	CMP + ATP <-> ADP + CDP	1
Cytidylate kinase	cmkA	UMP + ATP <-> ADP + UDP	1
Cytidylate kinase	cmkB	UMP + ATP <-> ADP + UDP	1
dTMP kinase	tmk	DTMP + ATP <-> ADP + DTDP	1
Uridylate kinase	pyrH	UMP + ATP <-> UDP + ADP	1
Uridylate kinase	pyrH	DUMP + ATP <-> DUDP + ADP	1
Thymidine (deoxyuridine) kinase	tdk	DU + ATP -> DUMP + ADP	1
Uracil phosphoribosyltransferase	upp	URA + PRPP -> UMP + PPI	1
Cytosine deaminase	codA	CYTS -> URA + NH3	1
Uridine kinase	udk	URI + GTP -> GDP + UMP	1
Cytodine kinase	udk	CYTD + GTP -> GDP + CMP	1
CMP glycosylase		CMP -> CYTS + R5P	0
Cytidine deaminase	cdd	CYTD -> URI + NH3	1
Thymidine (deoxyuridine) kinase	tdk	DT + ATP -> ADP + DTMP	1
dCTP deaminase	dcd	DCTP -> DUTP + NH3	1
Cytidine deaminase	cdd	DC -> NH3 + DU	1
5'-Nucleotidase	ushA	DUMP -> DU + PI	1
5'-Nucleotidase	ushA	DTMP -> DT + PI	1
5'-Nucleotidase	ushA	DAMP -> DA + PI	1
5'-Nucleotidase	ushA	DGMP -> DG + PI	1
5'-Nucleotidase	ushA	DCMP -> DC + PI	1

Enzyme	Gene	Reaction	E. coli genome
5'-Nucleotidase	ushA	CMP -> CYTD + PI	1
5'-Nucleotidase	ushA	AMP -> PI + ADN	1
5'-Nucleotidase	ushA	GMP -> PI + GSN	1
5'-Nucleotidase	ushA	IMP -> PI + INS	1
5'-Nucleotidase	ushA	XMP -> PI + XTSN	1
5'-Nucleotidase	ushA	UMP -> PI + URI	1
Ribonucleoside-diphosphate reductase	nrdAB	ADP + RTHIO -> DADP + OTHIO	2
Ribonucleoside-diphosphate reductase	nrdAB	GDP + RTHIO -> DGDP + OTHIO	2
Ribonucleoside-triphosphate reductase	nrdD	ATP + RTHIO -> DATP + OTHIO	1
Ribonucleoside-triphosphate reductase	nrdD	GTP + RTHIO -> DGTP + OTHIO	1
Ribonucleoside-diphosphate reductase	nrdAB	CDP + RTHIO -> DCDP + OTHIO	2
Ribonucleoside-diphosphate reductase II	nrdEF	CDP + RTHIO -> DCDP + OTHIO	2
Ribonucleoside-diphosphate reductase	nrdAB	UDP + RTHIO -> DUDP + OTHIO	2
Ribonucleoside-triphosphate reductase	nrdD	CTP + RTHIO -> DCTP + OTHIO	1
Ribonucleoside-triphosphate reductase	nrdD	UTP + RTHIO -> OTHIO + DUTP	1
dUTP pyrophosphatase	dut	DUTP -> PPI + DUMP	1
Thymidilate synthetase	thyA	DUMP + METTHF -> DHF + DTMP	1
Nucleoside triphosphatase	mutT	GTP -> GSN + 3 PI	1
Nucleoside triphosphatase	mutT	DGTP -> DG + 3 PI	1
Deoxyguanosinetriphosphate triphophohydrolase	dgt	DGTP -> DG + 3 PI	1
Deoxyguanosinetriphosphate triphophohydrolase	dgt	GTP -> GSN + 3 PI	1

Enzyme	Gene	Reaction	E. coli genome
Glycine cleavage system (Multi-component system)	gcvHTP, lpdA	GLY + THF + NAD -> METTHF + NADH + CO2 + NH3	4
Formyl tetrahydrofolate deformylase	purU	FTHF -> FOR + THF	1
Methylene tetrahydrofolate reductase	metF	METTHF + NADH -> NAD + MTHF	1
Methylene THF dehydrogenase	folD	METTHF + NADP <-> METHF + NADPH	1
Methenyl tetrahydrofolate cyclohydrolase	folD	METHF <-> FTHF	1
Acetyl-CoA carboxyltransferase	accABD	ACCOA + ATP + CO2 <-> MALCOA + ADP + PI	3
Malonyl-CoA-ACP transacylase	fabD	MALCOA + ACP <-> MALACP + COA	1
Malonyl-ACP decarboxylase	fabB	MALACP -> ACACP + CO2	1
Acetyl-CoA-ACP transacylase	fabH	ACACP + COA <-> ACCOA + ACP	1
Acyltransferase	pls	GL3P +0.035 C140ACP +0.102 C141ACP +0.717 C160ACP +0.142 C161ACP +1.004 C181ACP -> 2 ACP + PA	0
CDP-Diacylglycerol synthetase	cdsA	PA + CTP <-> CDPDG + PPI	1
CDP-Diacylglycerol pyrophosphatase	cdh	CDPDG -> CMP + PA	1
Phosphatidylserine synthase	pssA	CDPDG + SER <-> CMP + PS	1
Phosphatidylserine decarboxylase	psd	PS -> PE + CO2	1
Phosphatidylglycerol phosphate synthase	pgsA	CDPDG + GL3P <-> CMP + PGP	1
Phosphatidylglycerol phosphate phosphatase A	pgpA	PGP -> PI + PG	0
Phosphatidylglycerol phosphate phosphatase B	pgpB	PGP -> PI + PG	1
Cardiolipin synthase	cls	2 PG <-> CL + GL	1
Acetyl-CoA C-acetyltransferase	atoB	2 ACCOA <-> COA + AACCOA	1
Isoprenyl-pyrophosphate synthesis pathway		T3P1 + PYR + 2 NADPH + ATP -> IPPP + ADP + 2 NADP + CO2	0

Enzyme	Gene	Reaction	E. coli genome
Isoprenyl pyrophosphate isomerase		IPPP -> DMPP	0
Farnesyl pyrophosphate synthetase	ispA	DMPP + IPPP -> GPP + PPI	1
Geranyltransferase	ispA	GPP + IPPP -> FPP + PPI	1
Octaprenyl pyrophosphate synthase (5 reactions)	ispB	5 IPPP + FPP -> OPP + 5 PPI	1
Undecaprenyl pyrophosphate synthase (8 reactions)		8 IPPP + FPP -> UDPP + 8 PPI	0
Chorismate pyruvate-lyase	ubiC	CHOR -> 4HBZ + PYR	1
Hydroxybenzoate octaprenyltransferase	ubiA	4HBZ + OPP -> O4HBZ + PPI	1
Octaprenyl-hydroxybenzoate decarboxylase	ubiD, ubiX	O4HBZ -> CO2 + 2OPPP	1
2-Octaprenylphenol hydroxylase	ubiB	2OPPP + O2 -> 2O6H	1
Methylation reaction		2O6H + SAM -> 2OPMP + SAH	0
2-Octaprenyl-6-methoxyphenol hydroxylase	ubiH	2OPMP + O2 -> 2OPMB	1
2-Octaprenyl-6-methoxy-1,4-benzoquinone methylase	ubiE	2OPMB + SAM -> 2OPMMB + SAH	0
2-Octaprenyl-3-methyl-6-methoxy-1,4-benzoquinone hydroxylase	ubiF	2OPMMB + O2 -> 2OMHMB	0
3-Dimethylubiquinone 3-methyltransferase	ubiG	2OMHMB + SAM -> QH2 + SAH	1
Isochorismate synthase 1	menF	CHOR -> ICHOR	1
α-Ketoglutarate decarboxylase	menD	AKG + TPP -> SSALTPP + CO2	1
SHCHC synthase	menD	ICHOR + SSALTPP -> PYR + TPP + SHCHC	1
O-Succinylbenzoate-CoA synthase	menC	SHCHC -> OSB	1
O-Succinylbenzoic acid-CoA ligase	menE	OSB + ATP + COA -> OSBCOA + AMP + PPI	1
Naphthoate synthase	menB	OSBCOA -> DHNA + COA	1

Enzyme	Gene	Reaction	E. coli genome
1,4-Dihydroxy-2-naphthoate octaprenyltransferase	menA	DHNA + OPP -> DMK + PPI + CO2	1
S-Adenosylmethionine-2-DMK methyltransferase	menG	DMK + SAM -> MK + SAH	1
Isochorismate synthase 2	entC	CHOR -> ICHOR	1
Isochorismatase	entB	ICHOR <-> 23DHDHB + PYR	1
2,3-Dihydo-2,3-dihydroxybenzoate dehydrogenase	entA	23DHDHB + NAD <-> 23DHB + NADH	1
ATP-dependent activation of 2,3-dihydroxybenzoate	entE	23DHB + ATP <-> 23DHBA + PPI	1
ATP-dependent serine activating enzyme	entF	SER + ATP <-> SERA + PPI	1
Enterochelin synthetase	entD	3 SERA + 3 23DHBA -> ENTER + 6 AMP	1
GTP cyclohydrolase II	ribA	GTP -> D6RP5P + FOR + PPI	1
Pyrimidine deaminase	ribD	D6RP5P -> A6RP5P + NH3	1
Pyrimidine reductase	ribD	A6RP5P + NADPH -> A6RP5P2 + NADP	1
Pyrimidine phosphatase		A6RP5P2 -> A6RP + PI	0
3,4 Dihydroxy-2-butanone-4-phosphate synthase	ribB	RL5P -> DB4P + FOR	1
6,7-Dimethyl-8-ribityllumazine synthase	ribE	DB4P + A6RP -> D8RL + PI	1
Riboflavin synthase	ribH	2 D8RL -> RIBFLV + A6RP	1
Riboflavin kinase	ribF	RIBFLV + ATP -> FMN + ADP	1
FAD synthetase	ribF	FMN + ATP -> FAD + PPI	1
GTP cyclohydrolase I	folE	GTP -> FOR + AHTD	1
Dihydroneopterin triphosphate pyrophosphorylase	ntpA	AHTD -> PPI + DHPP	1
Nucleoside triphosphatase	mutT	AHTD -> DHP + 3 PI	1
Dihydroneopterin monophosphate dephosphorylase		DHPP -> DHP + PI	0

Enzyme	Gene	Reaction	E. coli genome
Dihydroneopterin aldolase	folB	DHP -> AHHMP + GLAL	1
6-Hydroxymethyl-7,8 dihydropterin pyrophosphokinase	folK	AHHMP + ATP -> AMP + AHHMD	1
Aminodeoxychorismate synthase	pabAB	CHOR + GLN -> ADCHOR + GLU	2
Aminodeoxychorismate lyase	pabC	ADCHOR -> PYR + PABA	1
Dihydropteroate synthase	folP	PABA + AHHMD -> PPI + DHPT	1
Dihydrofolate synthetase	folC	DHPT + ATP + GLU -> ADP + PI + DHF	1
Dihydrofolate reductase	folA	DHF + NADPH -> NADP + THF	1
Ketopentoate hydroxymethyl transferase	panB	OIVAL + METTHF -> AKP + THF	1
Ketopantoate reductase	panE	AKP + NADPH -> NADP + PANT	0
Acetohyoxyacid isomeroreductase	ilvC	AKP + NADPH -> NADP + PANT	1
Aspartate decarboxylase	panD	ASP -> CO2 + bALA	1
Pantoate-β-alanine ligase	panC	PANT + bALA + ATP -> AMP + PPI + PNTO	1
Pantothenate kinase	coaA	PNTO + ATP -> ADP + 4PPNTO	1
Phosphopantothenate-cysteine ligase		4PPNTO + CTP + CYS -> CMP + PPI + 4PPNCYS	0
Phosphopantothenate-cysteine decarboxylase		4PPNCYS -> CO2 + 4PPNTE	0
Phospho-pantethiene adenylyltransferase		4PPNTE + ATP -> PPI + DPCOA	0
DephosphoCoA kinase		DPCOA + ATP -> ADP + COA	0
ACP Synthase	acpS	COA -> PAP + ACP	1
Aspartate oxidase	nadB	ASP + FAD -> FADH + ISUCC	1
Quinolate synthase	nadA	ISUCC + T3P2 -> PI + QA	1

Enzyme	Gene	Reaction	E. coli genome
Quinolate phosphoribosyl transferase	nadC	QA + PRPP -> NAMN + CO2 + PPI	1
NAMN adenylyl transferase	nadD	NAMN + ATP -> PPI + NAAD	0
NAMN adenylyl transferase	nadD	NMN + ATP -> NAD + PPI	0
Deamido-NAD ammonia ligase	nadE	NAAD + ATP + NH3 -> NAD + AMP + PPI	1
NAD kinase	nadFG	NAD + ATP -> NADP + ADP	0
NADP phosphatase		NADP -> NAD + PI	0
DNA ligase	lig	NAD -> NMN + AMP	1
NMN amidohydrolase	pncC	NMN -> NAMN + NH3	0
NMN glycohydrolase (cytoplasmic)		NMN -> R5P + NAm	0
NAm amidohydrolase	pncA	NAm -> NAC + NH3	0
NAPRTase	pncB	NAC + PRPP + ATP -> NAMN + PPI + PI + ADP	1
NAD pyrophosphatase	pnuE	NADxt -> NMNxt + AMPxt	0
NMN permease	pnuC	NMNxt -> NMN	1
NMN glycohydrolase (membrane bound)		NMNxt -> R5P + NAm	0
Nicotinic acid uptake		NACxt -> NAC	0
GSA synthetase	hemM	GLU + ATP -> GTRNA + AMP + PPI	1
Glutamyl-tRNA synthetase	gltX	GLU + ATP -> GTRNA + AMP + PPI	1
Glutamyl-tRNA reductase	hemA	GTRNA + NADPH -> GSA + NADP	1
Glutamate-1-semialdehyde aminotransferase	hemL	GSA -> ALAV	1
Porphobilinogen synthase	hemB	8 ALAV -> 4 PBG	1
Hydroxymethylbilane synthase	hemC	4 PBG -> HMB + 4 NH3	1

Enzyme	Gene	Reaction	E. coli genome
Uroporphyrinogen III synthase	hemD	HMB -> UPRG	1
Uroporphyrin-III C-methyltransferase 1	hemX	SAM + UPRG -> SAH + PC2	1
Uroporphyrin-III C-methyltransferase 2	cysG	SAM + UPRG -> SAH + PC2	1
1,3-Dimethyluroporphyrinogen III dehydrogenase	cysG	PC2 + NAD -> NADH + SHCL	1
Siroheme ferrochelatase	cysG	SHCL -> SHEME	1
Uroporphyrinogen decarboxylase	hemE	UPRG -> 4 CO2 + CPP	1
Coproporphyrinogen oxidase, aerobic	hemF	O2 + CPP -> 2 CO2 + PPHG	2
Protoporphyrinogen oxidase	hemG	O2 + PPHG -> PPIX	2
Ferrochelatase	hemH	PPIX -> PTH	1
Heme O synthase	cyoE	PTH + FPP -> HO + PPI	1
8-Amino-7-oxononanoate synthase	bioF	ALA + CHCOA <-> CO2 + COA + AONA	1
Adenosylmethionine-8-amino-7-oxononanoate aminotransferase	bioA	SAM + AONA <-> SAMOB + DANNA	1
Dethiobiotin synthase	bioD	CO2 + DANNA + ATP <-> DTB + PI + ADP	1
Biotin synthase	bioB	DTB + CYS <-> BT	1
Glutamate-cysteine ligase	gshA	CYS + GLU + ATP -> GC + PI + ADP	1
Glutathione synthase	gshB	GLY + GC + ATP -> RGT + PI + ADP	1
Glutathione reductase	gor	NADPH + OGT <-> NADP + RGT	1
thiC protein	thiC	AIR -> AHM	1
HMP kinase	thiN	AHM + ATP -> AHMP + ADP	0
HMP-phosphate kinase	thiD	AHMP + ATP -> AHMPP + ADP	0

Enzyme	Gene	Reaction	E. coli genome
Hypothetical		T3P1 + PYR -> DTP	0
thiG protein	thiG	DTP + TYR + CYS -> THZ + HBA + CO2	1
thiE protein	thiE	DTP + TYR + CYS -> THZ + HBA + CO2	1
thiF protein	thiF	DTP + TYR + CYS -> THZ + HBA + CO2	1
thiH protein	thiH	DTP + TYR + CYS -> THZ + HBA + CO2	1
THZ kinase	thiM	THZ + ATP -> THZP + ADP	0
Thiamin phosphate synthase	thiB	THZP + AHMPP -> THMP + PPI	0
Thiamin kinase	thiK	THMP + ADP <-> THIAMIN + ATP	0
Thiamin phosphate kinase	thiL	THMP + ATP <-> TPP + ADP	0
Erythrose 4-phosphate dehydrogenase	epd	E4P + NAD <-> ER4P + NADH	1
Erythronate-4-phosphate dehydrogenase	pdxB	ER4P + NAD <-> OHB + NADH	1
Hypothetical transaminase/phosphoserine transaminase	serC	OHB + GLU <-> PHT + AKG	1
Pyridoxal-phosphate biosynthetic proteins pdxJ-pdxA	pdxAJ	PHT + DX5P -> P5P + CO2	2
Pyridoxine 5'-phosphate oxidase	pdxH	P5P + O2 <-> PL5P + H2O2	1
Threonine synthase	thrC	PHT -> 4HLT + PI	1
Hypothetical Enzyme		4HLT -> PYRDX	0
Pyridoxine kinase	pdxK	PYRDX + ATP -> P5P + ADP	1
Hypothetical Enzyme		P5P -> PYRDX + PI	0
Hypothetical Enzyme		PL5P -> PL + PI	0
Pyridoxine kinase	pdxK	PL + ATP -> PL5P + ADP	1

Enzyme	Gene	Reaction	E. coli genome
Pyridoxine 5'-phosphate oxidase	pdxH	PYRDX + O2 <-> PL + H2O2	1
Pyridoxine 5'-phosphate oxidase	pdxH	PL + O2 + NH3 <-> PDLA + H2O2	1
Pyridoxine kinase	pdxK	PDLA + ATP -> PDLA5P + ADP	1
Hypothetical Enzyme		PDLA5P -> PDLA + PI	0
Pyridoxine 5'-phosphate oxidase	pdxH	PDLA5P + O2 -> PL5P + H2O2 + NH3	1
Serine hydroxymethyltransferase (serine methylase)	glyA	PL5P + GLU -> PDLA5P + AKG	1
Serine hydroxymethyltransferase (serine methylase)	glyA	PL5P + ALA -> PDLA5P + PYR	1
Glutamine fructose-6-phosphate Transaminase	glmS	F6P + GLN -> GLU + GA6P	1
Phosphoglucosamine mutase	glmM	GA6P <-> GA1P	0
N-Acetylglucosamine-1-phosphate-uridyltransferase	glmU	UTP + GA1P + ACCOA -> UDPNAG + PPI + COA	1
UDP-N-acetylglucosamine acyltransferase	lpxA	C14OACP + UDPNAG -> ACP + UDPG2AA	1
UDP-3-O-acyl-N-acetylglucosamine deacetylase	lpxC	UDPG2AA -> UDPG2A + AC	1
UDP-3-O-(3-hydroxymyristoyl)glucosamine-acyltransferase	lpxD	UDPG2A + C14OACP -> ACP + UDPG23A	1
UDP-sugar hydrolase	ushA	UDPG23A -> UMP + LIPX	1
Lipid A disaccharide synthase	lpxB	LIPX + UDPG23A -> UDP + DISAC1P	1
Tetraacyldisaccharide 4' kinase		DISAC1P + ATP -> ADP + LIPIV	0
3-Deoxy-D-manno-octulosonic-acid transferase (KDO transferase)	kdtA	LIPIV + CMPKDO -> KDOLIPIV + CMP	1
3-Deoxy-D-manno-octulosonic-acid transferase (KDO transferase)	kdtA	KDOLIPIV + CMPKDO -> K2LIPIV + CMP	1
Endotoxin synthase	htrB, msbB	K2LIPIV + C14OACP + C12OACP -> LIPA + 2 ACP	2

Enzyme	Gene	Reaction	E. coli genome
3-Deoxy-D-manno-octulosonic-acid 8-phosphate synthase	kdsA	PEP + A5P -> KDOP + PI	1
3-Deoxy-D-manno-octulosonic-acid 8-phosphate phosphatase		KDOP -> KDO + PI	0
CMP-2-keto-3-deoxyoctonate synthesis	kdsB	KDO + CTP -> PPI + CMPKDO	1
ADP-L-glycero-D-mannoheptose-6-epimerase	lpcA, rfaED	S7P + ATP -> ADPHEP + PPI	1
UDP glucose-1-phosphate uridylyltransferase	galU, galF	G1P + UTP -> PPI + UDPG	2
Ethanolamine phosphotransferase		PE + CMP <-> CDPETN + DGR	0
Phosphatidate phosphatase		PA -> PI + DGR	0
Diacylglycerol kinase	dgkA	DGR + ATP -> ADP + PA	1
LPS Synthesis – truncated version of LPS (ref meid)	rfaLJIGFC	LIPA + 3 ADPHEP + 2 UDPG + 2 CDPETN + 3 CMPKDO -> LPS + 3 ADP + 2 UDP + 3 CMP + 2 CDP	6
UDP-N-acetylglucosamine-enolpyruvate transferase	murA	UDPNAG + PEP -> UDPNAGEP + PI	1
UDP-N-acetylglucosamine-enolpyruvate dehydrogenase	murB	UDPNAGEP + NADPH -> UDPNAM + NADP	1
UDP-N-acetylmuramate-alanine ligase	murC	UDPNAM + ALA + ATP -> ADP + PI + UDPNAMA	1
UDP-N-acetylmuramoylalanine-D-glutamate ligase	murD	UDPNAMA + DGLU + ATP -> UDPNAMAG + ADP + PI	1
UDP-N-acetylmuramoylalanyl-D-glutamate 2,6-diaminopimelate ligase	murE	UDPNAMAG + ATP + MDAP -> UNAGD + ADP + PI	1
D-Alanine-D-alanine adding enzyme	murF	UNAGD + ATP + AA -> UNAGDA + ADP + PI	1
Glutamate racemase	murI	GLU <-> DGLU	1
D-ala:D-ala ligases	ddlAB	2 DALA <-> AA	2

Enzyme	Gene	Reaction	E. coli genome 1
Phospho-N-acetylmuramoylpentapeptide transferase	mraY	UNAGDA·> UMP + PI + UNPTDO	1
N-Acetylglucosaminyl transferase	murG	UNPTDO + UDPNAG·> UDP + PEPTIDO	1
Arabinose (low affinity)	araE	ARABxt + HEXT <·> ARAB	1
Arabinose (high affinity)	araFGH	ARABxt + ATP·> ARAB + ADP + PI	3
Dihydroxyacetone		DHAxt + PEP·> T3P2 + PYR	0
Fructose	fruABF	FRUxt + PEP·> F1P + PYR	2
Fucose	fucP	FUCxt + HEXT <·> FUC	1
Galacitol	gatABC	GLTLxt + PEP·> GLT L1P + PYR	3
Galactose (low affinity)	galP	GLACxt + HEXT·> GLAC	1
Galactose (low affinity)	galP	GLCxt + HEXT·> GLC	1
Galactose (high affinity)	mglABC	GLACxt + ATP·> GLAC + ADP + PI	3
Glucitol	srlA1A2B	GLTxt + PEP·> GLT6P + PYR	3
Gluconate	gntST	GLCNxt + ATP·> GLCN + ADP + PI	1
Glucose	ptsG, crr	GLCxt + PEP·> G6P + PYR	2
Glycerol	glpF	GLxt <·> GL	1
Lactose	lacY	LCTSxt + HEXT <·> LCTS	1
Maltose	malX, crr, melEFGK, lamB	MLTxt + PEP·> MLT6P + PYR	7
Mannitol	mtlA, cmtAB	MNTxt + PEP·> MNT6P + PYR	3
Mannose	manXYZ, ptsPAI	MANxt + PEP·> MAN1P + PYR	6
Melibiose	melB	MELIxt + HEXT·> MELI	1

Enzyme	Gene	Reaction	E. coli genome
N-Acetylglucosamine	nagE, ptsN	NAG + PEP -> NAGP + PYR	2
Rhamnose	rhaT	RMNxt + ATP -> RMN + ADP + PI	1
Ribose	rbsABCD, xylH	RIBxt + ATP -> RIB + ADP + PI	5
Sucrose	scr	SUCxt + PEP -> SUC6P + PYR	0
Trehalose	treAB	TRExt + PEP -> TRE6P + PYR	2
Xylose (low affinity)	xylE	XYLxt + HEXT -> XYL	1
Xylose (high affinity)	xylFG, rbsB	XYLxt + ATP -> XYL + ADP + PI	3
Alanine	cycA	ALAxt + ATP -> ALA + ADP + PI	1
Arginine	artPMQJI, argT hisMQP	ARGxt + ATP -> ARG + ADP + PI	9
Asparagine (low Affinity)		ASNxt + HEXT <-> ASN	0
Asparagine (high Affinity)		ASNxt + ATP -> ASN + ADP + PI	0
Aspartate	gltP	ASPxt + HEXT -> ASP	1
Aspartate	gltJKL	ASPxt + ATP -> ASP + ADP + PI	3
Branched chain amino acid transport	brnQ	BCAAxt + HEXT <-> BCAA	1
Cysteine	not identified	CYSxt + ATP -> CYS + ADP + PI	0
D-Alanine	cycA	DALAxt + ATP -> DALA + ADP + PI	1
D-Alanine glycine permease	cycA	DALAxt + HEXT <-> DALA	1
D-Alanine glycine permease	cycA	DSERxt + HEXT <-> DSER	1
D-Alanine glycine permease	cycA	GLYxt + HEXT <-> GLY	1
Diaminopimelic acid		MDAPxt + ATP -> MDAP + ADP + PI	0
γ-Aminobutyrate transport	gabP	GABAxt + ATP -> GABA + ADP + PI	1

Enzyme	Gene	Reaction	E. coli genome
Glutamate	gltP	GLUxt + HEXT <-> GLU	1
Glutamate	gltS	GLUxt + HEXT <-> GLU	1
Glutamate	gltJKL	GLUxt + ATP -> GLU + ADP + PI	3
Glutamine	glnHPQ	GLNxt + ATP -> GLN + ADP + PI	3
Glycine	cycA, proVWX	GLYxt + ATP -> GLY + ADP + PI	4
Histidine	hisJMPQ	HISxt + ATP -> HIS + ADP + PI	4
Isoleucine	livJ	ILExt + ATP -> ILE + ADP + PI	1
Leucine	livHKM/livFGJ	LEUxt + ATP -> LEU + ADP + PI	6
Lysine	lysP	LYSxt + HEXT <-> LYS	1
Lysine	argT, hisMPQ	LYSxt + ATP -> LYS + ADP + PI	4
Lysine/Cadaverine	cadB	LYSxt + ATP -> LYS + ADP + PI	1
Methionine	metD	METxt + ATP -> MET + ADP + PI	0
Ornithine	argT, hisMPQ	ORNxt + ATP -> ORN + ADP + PI	4
Phenylalanine	aroP/mtr/pheP	PHExt + HEXT <-> PHE	3
Proline	putP, proPWX	PROxt + HEXT <-> PRO	4
Proline	cycA, proVW	PROxt + ATP -> PRO + ADP + PI	4
Putrescine	potEFHIG	PTRCxt + ATP -> PTRC + ADP + PI	5
Serine	sdaC	SERxt + HEXT <-> SER	1
Serine	cycA	SERxt + ATP -> SER + ADP + PI	1
Spermidine & putrescine	potABCD	SPMDxt + ATP -> SPMD + ADP + PI	4
Spermidine & putrescine	potABCD	PTRCxt + ATP -> PTRC + ADP + PI	4

Enzyme	Gene	Reaction	E. coli genome
Threonine	livJ	THRxt + ATP -> THR + ADP + PI	1
Threonine	tdcC	THRxt + HEXT <-> THR	1
Tryptophan	tnaB	TRPxt + HEXT <-> TRP	1
Tyrosine	tyrP	TYRxt + HEXT <-> TYR	1
Valine	livJ	VALxt + ATP -> VAL + ADP + PI	1
Dipeptide	dppABCDF	DIPEPxt + ATP -> DIPEP + ADP + PI	5
Oligopeptide	oppABCDF	OPEPxt + ATP -> OPEP + ADP + PI	5
Peptide	sapABD	PEPTxt + ATP -> PEPT + ADP + PI	3
Uracil	uraA	URAxt + HEXT -> URA	1
Nicotinamide mononucleotide transporter	pnuC	NMNxt + HEXT -> + NMN	1
Cytosine	codB	CYTSxt + HEXT -> CYTS	1
Adenine	purB	ADxt + HEXT -> AD	1
Guanine	gpt, hpt	GNxt <-> GN	2
Hypoxanthine	gpt, hpt	HYXNxt <-> HYXN	2
Xanthosine	xapB	XTSNxt <-> XTSN	1
Xanthine	gpt	XANxt <-> XAN	1
G-system	nupG	ADNxt + HEXT -> ADN	1
G-system	nupG	GSNxt + HEXT -> GSN	1
G-system	nupG	URIxt + HEXT -> URI	1
G-system	nupG	CYTDxt + HEXT -> CYTD	1
G-system (transports all nucleosides)	nupG	INSxt + HEXT -> INS	1

Enzyme	Gene	Reaction	E. coli genome
G-system	nupG	XTSNxt + HEXT·-> XTSN	1
G-system	nupG	DTxt + HEXT·-> DT	1
G-system	nupG	DINxt + HEXT·-> DIN	1
G-system	nupG	DGxt + HEXT·-> DG	1
G-system	nupG	DAxt + HEXT·-> DA	1
G-system	nupG	DCxt + HEXT·-> DC	1
G-system	nupG	DUxt + HEXT·-> DU	1
C-system	nupC	ADNxt + HEXT·-> ADN	1
C-system	nupC	URIxt + HEXT·-> URI	1
C-system	nupC	CYTDxt + HEXT·-> CYTD	1
C-system	nupC	DTxt + HEXT·-> DT	1
C-system	nupC	DAxt + HEXT·-> DA	1
C-system	nupC	DCxt + HEXT·-> DC	1
C-system	nupC	DUxt + HEXT·-> DU	1
Nucleosides and deoxynucleoside	tsx	ADNxt + HEXT·-> ADN	1
Nucleosides and deoxynucleoside	tsx	GSNxt + HEXT·-> GSN	1
Nucleosides and deoxynucleoside	tsx	URIxt + HEXT·-> URI	1
Nucleosides and deoxynucleoside	tsx	CYTDxt + HEXT·-> CYTD	1
Nucleosides and deoxynucleoside	tsx	INSxt + HEXT·-> INS	1
Nucleosides and deoxynucleoside	tsx	XTSNxt + HEXT·-> XTSN	1
Nucleosides and deoxynucleoside	tsx	DTxt + HEXT·-> DT	1

Enzyme	Gene	Reaction	E. coli genome
Nucleosides and deoxynucleoside	tsx	DINxt + HEXT -> DIN	1
Nucleosides and deoxynucleoside	tsx	DGxt + HEXT -> DG	1
Nucleosides and deoxynucleoside	tsx	DAxt + HEXT -> DA	1
Nucleosides and deoxynucleoside	tsx	DCxt + HEXT -> DC	1
Nucleosides and deoxynucleoside	tsx	DUxt + HEXT -> DU	1
Acetate transport		ACxt + HEXT <-> AC	0
Lactate transport		LACxt + HEXT <-> LAC	0
L-Lactate	lldP	LLACxt + HEXT <-> LLAC	1
Formate transport	focA	FORxt <-> FOR	1
Ethanol transport		ETHxt + HEXT <-> ETH	0
Succinate transport	dcuAB	SUCCxt + HEXT <-> SUCC	2
Pyruvate transport		PYRxt + HEXT <-> PYR	0
Ammonia transport	amtB	NH3xt + HEXT <-> NH3	1
Potassium transport	kdpABC	Kxt + ATP -> K + ADP + PI	3
Potassium transport	trkAEHG	Kxt + HEXT <-> K	3
Sulfate transport	cysPTUWAZ, sbp	SLFxt + ATP -> SLF + ADP + PI	7
Phosphate transport	pstABCS	PIxt + ATP -> ADP + 2 PI	4
Phosphate transport	pitAB	PIxt + HEXT <-> PI	2
Glycerol-3-phosphate	glpT, ugpABCE	GL3Pxt + PI -> GL3P	5
Dicarboxylates	dcuAB, dctA	SUCCxt + HEXT <-> SUCC	3
Dicarboxylates	dcuAB, dctA	FUMxt + HEXT <-> FUM	3

Enzyme	Gene	Reaction	E. coli genome
Dicarboxylates	dcuAB, dctA	MALxt + HEXT <-> MAL	3
Dicarboxylates	dcuAB, dctA	ASPxt + HEXT <-> ASP	3
Fatty acid transport	fadL	C140xt -> C140	1
Fatty acid transport	fadL	C160xt -> C160	1
Fatty acid transport	fadL	C180xt -> C180	1
α-Ketoglutarate	kgtP	AKGxt + HEXT <-> AKG	1
Na/H antiporter	nhaABC	NAxt + <-> NA + HEXT	2
Na/H antiporter	chaABC	NAxt + <-> NA + HEXT	3
Pantothenate	panF	PNTOxt + HEXT <-> PNTO	1
Sialic acid permease	nanT	SLAxt + ATP -> SLA + ADP + PI	1
Oxygen transport		O2xt <-> O2	0
Carbon dioxide transport		CO2xt <-> CO2	0
Urea transport		UREAxt + 2 HEXT <-> UREA	0
ATP drain flux for constant maintenance requirements		ATP -> ADP + PI	0
Glyceraldehyde transport	gulP	GLALxt <-> GLAL	0
Acetaldehyde transport		ACALxt <-> ACAL	0

Table 2

Comparison of the predicted mutant growth characteristics from the gene deletion study to published experimental results with single and double mutants.

Gene	Glucose (in vivo|in silico)	Glycerol (in vivo|in silico)	Succinate (in vivo|in silico)	Acetate (in vivo|in silico)
aceEF	-|+			
aceA				-|-
aceB				-|-
ackA				+|+
acs				+|+
acn	-|-	-|-	-|-	-|-
cyd	+|+			
cyo	+|+			
eno	-|+	-|+	-|-	-|-
fba	-|+			
fbp	+|+	-|-	-|-	-|-
gap	-|-	-|-	-|-	-|-
gltA	-|-	-|-	-|-	-|-
gnd	+|+			
idh	-|-	-|-	-|-	-|-
ndh	+|+	+|+		
nuo	+|+	+|+		
pfk	-|+			
pgi	+|+	+|+		
pgk	-|-	-|-	-|-	-|-
pgl	+|+			
pntAB	+|+	+|+	+|+	+|+
glk	+|+			
ppc	±|+	-|+	+|+	+|+
pta				+|+
pts	+|+			
pyk	+|+			
rpi	-|-	-|-	-|-	-|-
sdhABCD	+|+			
tpi	-|+	-|-	-|-	-|-

Gene	Glucose (*in vivo*|*in silico*)	Glycerol (*in vivo*|*in silico*)	Succinate (*in vivo*|*in silico*)	Acetate (*in vivo*|*in silico*)
unc	+/+		-/-	-/-
zwf	+/+			
sucAD	+/+			
zwf, pnt	+/+			
pck, mez			-/-	-/-
pck, pps			-/-	-/-
pgi, zwf	-/-			
pgi, gnd	-/-			
pta,acs				-/-
tktA, tktB	-/-			

Results are scored as + or - meaning growth or no growth determined from *in vivo* / *in silico* data. In 73 of 80 cases the *in silico* behavior is the same as the experimentally observed behavior.

WHAT IS CLAIMED IS:

1.   A method for determining the genome specific stoichiometric matrix of an organism, comprising:

providing the nucleotide sequence of a metabolic gene in the organism;

identifying the open reading frame of the metabolic gene;

assigning a function to the metabolic gene based on its nucleotide or amino acid homology to other,

known metabolic genes;

determining the metabolic genotype of the organism based on the assigned function of the metabolic gene; and

determining the genome specific stoichiometric matrix for the organism.

2.   The method of Claim 1, further comprising determining a phenotype of the organism.

3.   The method of Claim 2, wherein determining the phenotype of the organism comprises analyzing the consequences of reduction or addition to the composition of the metabolic genotype.

4.   The method of Claim 2, further comprising identifying lethal genetic deletions.

5.   The method of Claim 4 further comprising determining the effectiveness of a drug through analysis of the lethal genetic deletions.

6.   The method of Claim 1, further comprising determining the minimal media composition required to sustain growth of the organism.

7.   The method of Claim 1, further comprising determining an optimal media composition for growing the organism.

8.   The method of Claim 1, further comprising determining the most advantageous complement of genes in the organism necessary to sustain growth in a particular environmental condition.

9.   The method of Claim 1, wherein the organism is *Escherichia Coli*.

10.   The method of Claim 1, comprising the use of a Flux Based Analysis on the stoichiometric matrix.

11.   The method of Claim 1, comprising adding biochemical information for a metabolic gene to the stoichiometric matrix.

12.   A method for determining a potential genetic target for a drug that kills an organism, comprising:

providing the nucleotide sequence of a metabolic gene in the organism;

identifying the open reading frame of the metabolic gene;

assigning a function to the metabolic gene based on its nucleotide or amino acid homology to other, known metabolic genes;

determining whether the metabolic gene is required for growth of the organism;

repeating the providing, identifying, assigning and determining steps for other metabolic genes of the organism; and

selecting a gene that is required for growth of the organism as a target for the drug.

13.   The method of Claim 12, wherein the organism is *Escherichia coli*.

14.   The method of Claim 12, comprising performing a Flux Based Analysis of a stoichiometric matrix from the organism.

15.   The method of Claim 12, comprising the use of biochemical information on the metabolic gene to determine whether it is required for growth of the organism.

16.   A computer system comprising a memory having instructions that when executed perform the steps of:

        providing the nucleotide sequence of a metabolic gene in an organism;

        identifying the open reading frame of the metabolic gene;

        assigning a function to the metabolic gene based on its nucleotide or amino acid homology to other, known metabolic genes;

        determining the metabolic genotype of the organism based on the assigned function of the unknown metabolic gene; and

        determining the genome specific stoichiometric matrix for the organism.

17.   The computer system of Claim 16, wherein said memory is selected from the group consisting of: a hard disk, optical memory, Random Access Memory, Read Only Memory and Flash Memory.

18.   The computer system of Claim 16, wherein said computer system is based on an Intel® microprocessor.

19.   The computer system of Claim 16, wherein the organism is *Escherichia Coli.*

20.   The computer system of Claim 16, further comprising instructions that when executed perform the method of identifying lethal genetic deletions for the organism.

21.   The computer system of Claim 16, comprising instructions, that when executed, add biochemical information on a metabolic gene to the stoichiometric matrix.

22.   A method for representing a living organism in a computer system, comprising:

        providing the nucleotide sequence of a metabolic gene in the organism;

        identifying the open reading frame of the metabolic gene;

        assigning a function to the metabolic gene based on its nucleotide or amino acid homology to other, known metabolic genes;

        determining the metabolic genotype of the organism based on the assigned function of the metabolic gene;

        determining the genome specific stoichiometric matrix for the organism; and

        storing the genome specific stoichiometric matrix in a memory of the computer.

23.   The method of Claim 22, wherein the organism is *Escherichia coli.*

24.   The method of Claim 22, comprising the use of Flux Based Analysis to analyze the stoichiometric matrix.

25.   The method of Claim 22, comprising adding biochemical information on a metabolic gene to determine the metabolic genotype of the organism.

26.   The method of Claim 22, comprising calculating the genome specific stoichiometric matrix using Flux Based Analysis.

27.   A genome specific stoichiometric matrix representing the metabolism of a living organism, produced by a process comprising:

5   providing the nucleotide sequence of a metabolic gene in the organism;

                     identifying the open reading frame of the metabolic gene;

                     assigning a function to the metabolic gene based on its nucleotide or amino acid homology to other, known metabolic genes;

                     determining the metabolic genotype of the organism based on the assigned function of the
10   metabolic gene; and

                     determining the genome specific stoichiometric matrix for the organism.

28.   The stoichiometric matrix of Claim 1, wherein the organism is *Escherichia Coli*.

29.   The stoichiometric matrix of Claim 1, wherein the stoichiometric matrix is determined using Flux Based Analysis.

15   30.   The stoichiometric matrix of Claim 1, produced by the process of adding biochemical information for the metabolic gene.
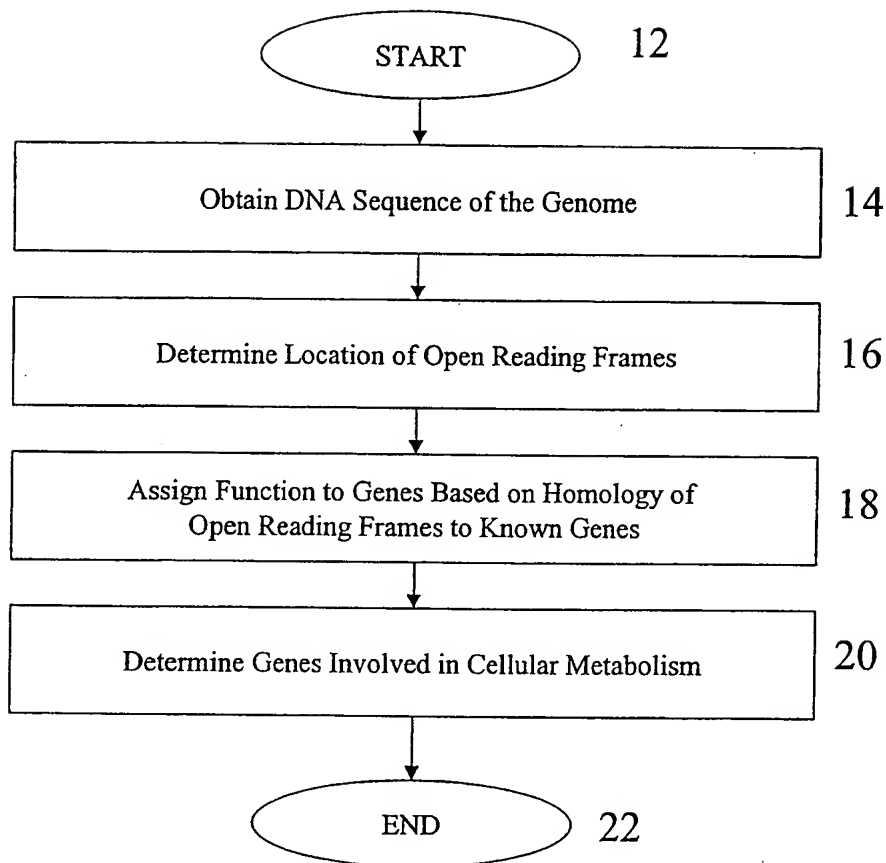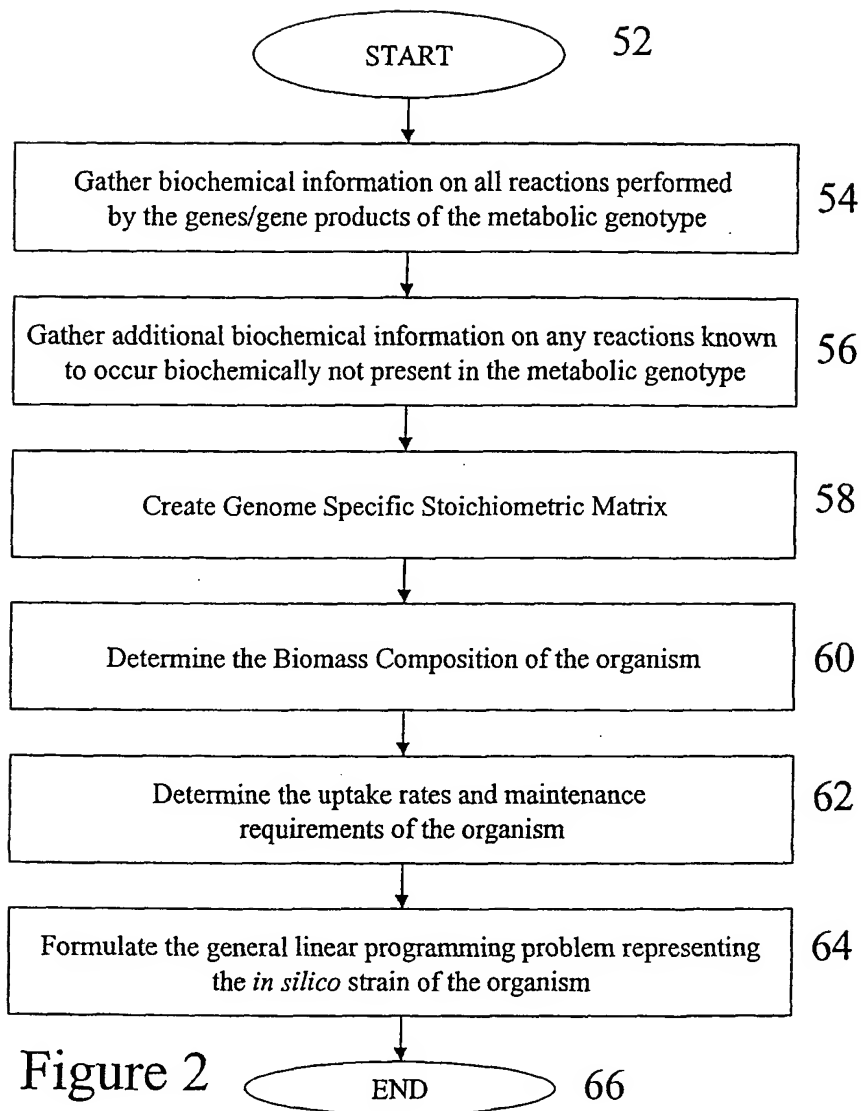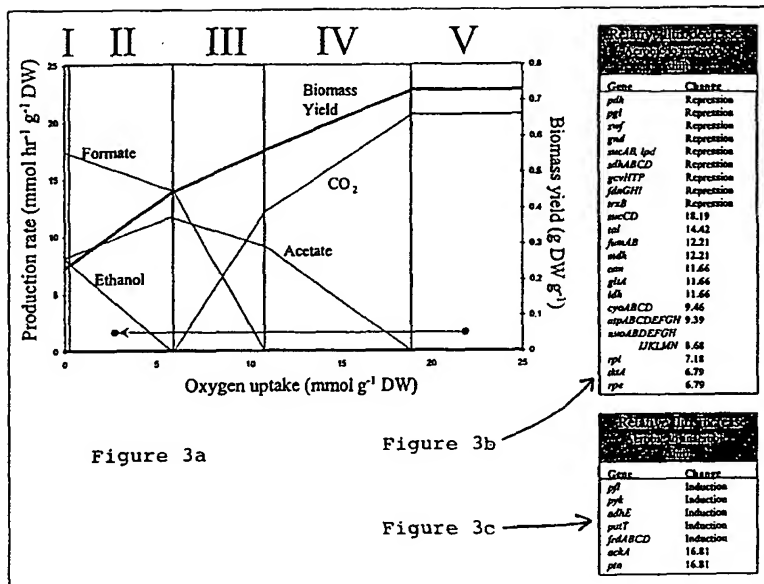
Figure 1

Figure 2

Figur 3

Figure 3a

Figure 3b

Figure 3c

CORRECTED VERSION

(51) International Patent Classification⁷: C12Q 1/68, G06F 19/00

(21) International Application Number: PCT/US00/02882

(22) International Filing Date: 2 February 2000 (02.02.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/243,022 2 February 1999 (02.02.1999) US

(71) Applicant and
(72) Inventor: PALSSON, Bernhard [US/US]; 730 Fern Glen, La Jolla, CA 92037 (US).

(74) Agent: HUNT, Dale, C.; 16th Floor, 620 Newport Center Drive, Newport Beach, CA 92660 (US).

(81) Designated States *(national)*: AE, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR (utility model), KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *with amended claims*

(88) Date of publication of the international search report:
10 May 2001

(48) Date of publication of this corrected version:
7 September 2001

(15) Information about Correction:
see PCT Gazette No. 36/2001 of 7 September 2001, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS FOR IDENTIFYING DRUG TARGETS BASED ON GENOMIC SEQUENCE DATA

(57) Abstract: This invention provides a computational approach to identifying potential antibacterial drug targets based on a genome sequence and its annotation. Starting from a fully sequenced genome, open reading frame assignments are made which determine the metabolic genotype for the organism. The metabolic genotype, and more specifically its stoichiometric matrix, are analyzed using flux balance analysis to assess the effects of genetic deletions on the fitness of the organism and its ability to produce essential biomolecules required for growth.

METHODS FOR IDENTIFYING DRUG TARGETS
BASED ON GENOMIC SEQUENCE DATA

Background of the Invention

Field of the Invention

5   This invention relates to methods for identifying drug targets based on genomic sequence data. More specifically, this invention relates to systems and methods for determining suitable molecular targets for the directed development of antimicrobial agents.

Description of the Related Art

          Infectious disease is on a rapid rise and threatens to regain its status as a major health problem. Prior to the

10   discovery of antibiotics in the 1930s, infectious disease was a major cause of death. Further discoveries, development, and mass production of antibiotics throughout the 1940s and 1950s dramatically reduced deaths from microbial infections to a level where they effectively no longer represented a major threat in developed countries.

          Over the years antibiotics have been liberally prescribed and the strong selection pressure that this represents has led to the emergence of antibiotic resistant strains of many serious human pathogens. In some cases

15   selected antibiotics, such as vancomycin, literally represent the last line of defense against certain pathogenic bacteria such as *Staphylococcus*. The possibility for staphylococci to acquire vancomycin resistance through exchange of genetic material with enterococci, which are commonly resistant to vancomycin, is a serious issue of concern to health care specialists. The pharmaceutical industry continues its search for new antimicrobial compounds, which is a lengthy and tedious, but very important process. The rate of development and introduction of new antibiotics appears to no

20   longer be able to keep up with the evolution of new antibiotic resistant organisms. The rapid emergence of antibiotic resistant organisms threatens to lead to a serious widespread health care concern.

          The basis of antimicrobial chemotherapy is to selectively kill the microbe with minimal, and ideally no, harm to normal human cells and tissues. Therefore, ideal targets for antibacterial action are biochemical processes that are unique to bacteria, or those that are sufficiently different from the corresponding mammalian processes to allow

25   acceptable discrimination between the two. For effective antibiotic action it is clear that a vital target must exist in the bacterial cell and that the antibiotic be delivered to the target in an active form. Therefore resistance to an antibiotic can arise from: (i) chemical destruction or inactivation of the antibiotic; (ii) alteration of the target site to reduce or eliminate effective antibiotic binding; (iii) blocking antibiotic entry into the cell, or rapid removal from the cell after entry; and (iv) replacing the metabolic step inhibited by the antibiotic.

30   Thus, it is time to fundamentally re-examine the philosophy of microbial killing strategies and develop new paradigms. One such paradigm is a holistic view of cellular metabolism. The identification of "sensitive" metabolic steps in attaining the necessary metabolic flux distributions to support growth and survival that can be attacked to weaken or destroy a microbe, need not be localized to a single biochemical reaction or cellular process. Rather, different cellular targets that need not be intimately related in the metabolic topology could be chosen based on the

35   concerted effect the loss of each of these functions would have on metabolism.

A similar strategy with viral infections has recently proved successful. It has been shown that "cocktails" of different drugs that target different biochemical processes provide enhanced success in fighting against HIV infection. Such a paradigm shift is possible only if the necessary biological information as well as appropriate methods of rational analysis are available. Recent advances in the field of genomics and bioinformatics, in addition to mathematical
5   modeling, offer the possibility to realize this approach.

At present, the field of microbial genetics is entering a new era where the genomes of several microorganisms are being completely sequenced. It is expected that in a decade, or so, the nucleotide sequences of the genomes of all the major human pathogens will be completely determined. The sequencing of the genomes of pathogens such as *Haemophilus influenzae* has allowed researchers to compare the homology of proteins encoded by
10   the open reading frames (ORFs) with those of *Escherichia coli*, resulting in valuable insight into the *H. influenzae* metabolic features. Similar analyses, such as those performed with *H. influenzae*, will provide details of metabolism spanning the hierarchy of metabolic regulation from bacterial genomes to phenotypes.

These developments provide exciting new opportunities to carry out conceptual experiments *in silico* to analyze different aspects of microbial metabolism and its regulation. Further, the synthesis of whole-cell models is
15   made possible. Such models can account for each and every single metabolic reaction and thus enable the analysis of their role in overall cell function.   To implement such analysis, however, a mathematical modeling and simulation framework is needed which can incorporate the extensive metabolic detail but still retain computational tractability. Fortunately, rigorous and tractable mathematical methods have been developed for the required systems analysis of metabolism.

20   A mathematical approach that is well suited to account for genomic detail and avoid reliance on kinetic complexity has been developed based on well-known stoichiometry of metabolic reactions. This approach is based on metabolic flux balancing in a metabolic steady state. The history of flux balance models for metabolic analyses is relatively short. It has been applied to metabolic networks, and the study of adipocyte metabolism. Acetate secretion from *E. coli* under ATP maximization conditions and ethanol secretion by yeast have also been investigated using this
25   approach.

The complete sequencing of a bacterial genome and ORF assignment provides the information needed to determine the relevant metabolic reactions that constitute metabolism in a particular organism.   Thus a flux-balance model can be formulated and several metabolic analyses can be performed to extract metabolic characteristics for a particular organism. The flux balance approach can be easily applied to systematically simulate the effect of single, as
30   well as multiple, gene deletions. This analysis will provide a list of sensitive enzymes that could be potential antimicrobial targets.

The need to consider a new paradigm for dealing with the emerging problem of antibiotic resistant pathogens is a problem of vital importance. The route towards the design of new antimicrobial agents must proceed along directions that are different from those of the past. The rapid growth in bioinformatics has provided a wealth of
35   biochemical and genetic information that can be used to synthesize complete representations of cellular metabolism. These models can be analyzed with relative computational ease through flux-balance models and visual computing

techniques. The ability to analyze the global metabolic network and understand the robustness and sensitivity of its regulation under various growth conditions offers promise in developing novel methods of antimicrobial chemotherapy.

In one example, Pramanik et al. described a stoichiometric model of *E. coli* metabolism using flux-balance modeling techniques (*Stoichiometric Model of Escherichia coli Metabolism: Incorporation of Growth-Rate Dependent*
5   *Biomass Composition and Mechanistic Energy Requirements*, Biotechnology and Bioengineering, Vol. 56, No. 4, November 20, 1997). However, the analytical methods described by Pramanik, et al. can only be used for situations in which biochemical knowledge exists for the reactions occurring within an organism. Pramanik, et al. produced a metabolic model of metabolism for *E. coli* based on biochemical information rather than genomic data since the metabolic genes and related reactions for *E. coli* had already been well studied and characterized. Thus, this method is
10   inapplicable to determining a metabolic model for organisms for which little or no biochemical information on metabolic enzymes and genes is known. It can be envisioned that in the future the only information we may have regarding an emerging pathogen is its genomic sequence. What is needed in the art is a system and method for determining and analyzing the entire metabolic network of organisms whose metabolic reactions have not yet been determined from biochemical assays. The present invention provides such a system.

15   Summary of the Invention

This invention relates to constructing metabolic genotypes and genome specific stoichiometric matrices from genome annotation data. The functions of the metabolic genes in the target organism are determined by homology searches against databases of genes from similar organisms. Once a potential function is assigned to each metabolic gene of the target organism, the resulting data is analyzed. In one embodiment, each gene is subjected to a flux-
20   balance analysis to assess the effects of genetic deletions on the ability of the target organism to produce essential biomolecules necessary for its growth. Thus, the invention provides a high-throughput computational method to screen for genetic deletions which adversely affect the growth capabilities of fully sequenced organisms.

Embodiments of this invention also provide a computational, as opposed to an experimental, method for the rapid screening of genes and their gene products as potential drug targets to inhibit an organism's growth. This
25   invention utilizes the genome sequence, the annotation data, and the biomass requirements of an organism to construct genomically complete metabolic genotypes and genome-specific stoichiometric matrices. These stoichiometric matrices are analyzed using a flux-balance analysis. This invention describes how to assess the affects of genetic deletions on the fitness and productive capabilities of an organism under given environmental and genetic conditions.

30   Construction of a genome-specific stoichiometric matrix from genomic annotation data is illustrated along with applying flux-balance analysis to study the properties of the stoichiometric matrix, and hence the metabolic genotype of the organism. By limiting the constraints on various fluxes and altering the environmental inputs to the metabolic network, genetic deletions may be analyzed for their affects on growth. This invention is embodied in a software application that can be used to create the stoichiometric matrix for a fully sequenced and annotated genome.
35   Additionally, the software application can be used to further analyze and manipulate the network so as to predict the ability of an organism to produce biomolecules necessary for growth, thus, essentially simulating a genetic deletion.

<u>Brief Description of the Drawings</u>

Figure 1 is a flow diagram illustrating one procedure for creating metabolic genotypes from genomic sequence data for any organism.

Figure 2 is a flow diagram illustrating one procedure for producing in silico microbial strains from the metabolic genotypes created by the method of Figure 1, along with additional biochemical and microbiological data.

Figure 3 is a graph illustrating a prediction of genome scale shifts in transcription. The graph shows the different phases of the metabolic response to varying oxygen availability, starting from completely aerobic to completely anaerobic in *E. coli*. The predicted changes in expression pattern between phases II and V are indicated.

<u>Detailed Description of the Preferred Embodiment</u>

This invention relates to systems and methods for utilizing genome annotation data to construct a stoichiometric matrix representing most of all of the metabolic reactions that occur within an organism. Using these systems and methods, the properties of this matrix can be studied under conditions simulating genetic deletions in order to predict the affect of a particular gene on the fitness of the organism. Moreover, genes that are vital to the growth of an organism can be found by selectively removing various genes from the stoichiometric matrix and thereafter analyzing whether an organism with this genetic makeup could survive. Analysis of these lethal genetic mutations is useful for identifying potential genetic targets for anti-microbial drugs.

It should be noted that the systems and methods described herein can be implemented on any conventional host computer system, such as those based on Intel® microprocessors and running Microsoft Windows operating systems. Other systems, such as those using the UNIX or LINUX operating system and based on IBM®, DEC® or Motorola® microprocessors are also contemplated. The systems and methods described herein can also be implemented to run on client-server systems and wide-area networks, such as the Internet.

Software to implement the system can be written in any well-known computer language, such as Java, C, C++, Visual Basic, FORTRAN or COBOL and compiled using any well-known compatible compiler.

The software of the invention normally runs from instructions stored in a memory on the host computer system. Such a memory can be a hard disk, Random Access Memory, Read Only Memory and Flash Memory. Other types of memories are also contemplated to function within the scope of the invention.

A process 10 for producing metabolic genotypes from an organism is shown in Figure 1. Beginning at a start state 12, the process 10 then moves to a state 14 to obtain the genomic DNA sequence of an organism. The nucleotide sequence of the genomic DNA can be rapidly determined for an organism with a genome size on the order of a few million base pairs. One method for obtaining the nucleotide sequences in a genome is through commercial gene databases. Many gene sequences are available on-line through a number of sites (see, for example, www.tigr.org) and can easily be downloaded from the Internet. Currently, there are 16 microbial genomes that have been fully sequenced and are publicly available, with countless others held in proprietary databases. It is expected that a number of other organisms, including pathogenic organisms will be found in nature for which little experimental information, except for its genome sequence, will be available.

Once the nucleotide sequence of the entire genomic DNA in the target organism has been obtained at state 14, the coding regions, also known as open reading frames, are determined at a state 16. Using existing computer algorithms, the location of open reading frames that encode genes from within the genome can be determined. For example, to identify the proper location, strand, and reading frame of an open reading frame one can perform a gene
5   search by signal (promoters, ribosomal binding sites, etc.) or by content (positional base frequencies, codon preference). Computer programs for determining open reading frames are available, for example, by the University of Wisconsin Genetics Computer Group and the National Center for Biotechnology Information.

After the location of the open reading frames have been determined at state 16, the process 10 moves to state 18 to assign a function to the protein encoded by the open reading frame. The discovery that an open reading
10   frame or gene has sequence homology to a gene coding for a protein of known function, or family of proteins of known function, can provide the first clues about the gene and it's related protein's function. After the locations of the open reading frames have been determined in the genomic DNA from the target organism, well-established algorithms (i.e. the Basic Local Alignment Search Tool (BLAST) and the FAST family of programs can be used to determine the extent of similarity between a given sequence and gene/protein sequences deposited in worldwide genetic databases. If a
15   coding region from a gene in the target organism is homologous to a gene within one of the sequence databases, the open reading frame is assigned a function similar to the homologously matched gene. Thus, the functions of nearly the entire gene complement or genotype of an organism can be determined so long as homologous genes have already been discovered.

All of the genes involved in metabolic reactions and functions in a cell comprise only a subset of the
20   genotype. This subset of genes is referred to as the metabolic genotype of a particular organism. Thus, the metabolic genotype of an organism includes most or all of the genes involved in the organism's metabolism. The gene products produced from the set of metabolic genes in the metabolic genotype carry out all or most of the enzymatic reactions and transport reactions known to occur within the target organism as determined from the genomic sequence.

To begin the selection of this subset of genes, one can simply search through the list of functional gene
25   assignments from state 18 to find genes involved in cellular metabolism. This would include genes involved in central metabolism, amino acid metabolism, nucleotide metabolism, fatty acid and lipid metabolism, carbohydrate assimilation, vitamin and cofactor biosynthesis, energy and redox generation, etc. This subset is generated at a state 20. The process 10 of determining metabolic genotype of the target organism from genomic data then terminates at an end stage 22.

30   Referring now to Figure 2, the process 50 of producing a computer model of an organism. This process is also known as producing *in silico* microbial strains. The process 50 begins at a start state 52 (same as end state 22 of process 10) and then moves to a state 54 wherein biochemical information is gathered for the reactions performed by each metabolic gene product for each of the genes in the metabolic genotype determined from process 10.

For each gene in the metabolic genotype, the substrates and products, as well as the stoichiometry of any
35   and all reactions performed by the gene product of each gene can be determined by reference to the biochemical

literature. This includes information regarding the irreverisble or reversible nature of the reactions. The stoichiometry of each reaction provides the molecular ratios in which reactants are converted into products.

Potentially, there may still remain a few reactions in cellular metabolism which are known to occur from *in vitro* assays and experimental data. These would include well characterized reactions for which a gene or protein has yet to be identified, or was unidentified from the genome sequencing and functional assignment of state 14 and 18. This would also include the transport of metabolites into or out of the cell by uncharacterized genes related to transport. Thus one reason for the missing gene information may be due to a lack of characterization of the actual gene that performs a known biochemical conversion. Therefore upon careful review of existing biochemical literature and available experimental data, additional metabolic reactions can be added to the list of metabolic reactions determined from the metabolic genotype from state 54 at a state 56. This would include information regarding the substrates, products, reversibilty/irreversibility, and stoichiometry of the reactions.

All of the information obtained at states 54 and 56 regarding reactions and their stoichiometry can be represented in a matrix format typically referred to as a stoichiometric matrix. Each column in the matrix corresponds to a given reaction or flux, and each row corresponds to the different metabolites involved in the given reaction/flux. Reversible reactions may either be represented as one reaction that operates in both the forward and reverse direction or be decomposed into one forward reaction and one backward reaction in which case all fluxes can only take on positive values. Thus, a given position in the matrix describes the stoichiometric participation of a metabolite (listed in the given row) in a particular flux of interest (listed in the given column). Together all of the columns of the genome specific stoichiometric matrix represent all of the chemical conversions and cellular transport processes that are determined to be present in the organism. This includes all internal fluxes and so called exchange fluxes operating within the metabolic network. Thus, the process 50 moves to a state 58 in order to formulate all of the cellular reactions together in a genome specific stoichiometric matrix. The resulting genome specific stoichiometric matrix is a fundamental representation of a genomically and biochemically defined genotype.

After the genome specific stoichiometric matrix is defined at state 58, the metabolic demands placed on the organism are calculated. The metabolic demands can be readily determined from the dry weight composition of the cell. In the case of well-studied organisms such as *Escherichia coli* and *Bacillus subtilis*, the dry weight composition is available in the published literature. However, in some cases it will be necessary to experimentally determine the dry weight composition of the cell for the organism in question. This can be accomplished with varying degrees of accuracy. The first attempt would measure the RNA, DNA, protein, and lipid fractions of the cell. A more detailed analysis would also provide the specific fraction of nucleotides, amino acids, etc. The process 50 moves to state 60 for the determination of the biomass composition of the target organism.

The process 50 then moves to state 62 to perform several experiments that determine the uptake rates and maintenance requirements for the organism. Microbiological experiments can be carried out to determine the uptake rates for many of the metabolites that are transported into the cell. The uptake rate is determined by measuring the depletion of the substrate from the growth media. The measurement of the biomass at each point is also required, in order to determine the uptake rate per unit biomass. The maintenance requirements can be determined from a

chemostat experiment. The glucose uptake rate is plotted versus the growth rate, and the y-intercept is interpreted as the non-growth associated maintenance requirements. The growth associated maintenance requirements are determined by fitting the model results to the experimentally determined points in the growth rate versus glucose uptake rate plot.

5   Next, the process 50 moves to a state 64 wherein information regarding the metabolic demands and uptake rates obtained at state 62 are combined with the genome specific stoichiometric matrix of step 8 together fully define the metabolic system using flux balance analysis (FBA). This is an approach well suited to account for genomic detail as it has been developed based on the well-known stoichiometry of metabolic reactions.

The time constants characterizing metabolic transients and/or metabolic reactions are typically very rapid, on
10   the order of milli-seconds to seconds, compared to the time constants of cell growth on the order of hours to days.Thus, the transient mass balances can be simplified to only consider the steady state behavior. Eliminating the time derivatives obtained from dynamic mass balances around every metabolite in the metabolic system, yields the system of linear equations represented in matrix notation,

$$S \bullet v = 0 \qquad\qquad \text{Equation 1}$$

15   where S refers to the stoichiometric matrix of the system, and v is the flux vector. This equation simply states that over long times, the formation fluxes of a metabolite must be balanced by the degradation fluxes. Otherwise, significant amounts of the metabolite will accumulate inside the metabolic network. Applying equation 1 to our system we let S now represent the genome specific stoichiometric matrix

To determine the metabolic capabilities of a defined metabolic genotype Equation 1 is solved for the
20   metabolic fluxes and the internal metabolic reactions, v, while imposing constraints on the activity of these fluxes. Typically the number of metabolic fluxes is greater than the number of mass balances (i.e., $m > n$) resulting in a plurality of feasible flux distributions that satisfy Equation 1 and any constraints placed on the fluxes of the system. This range of solutions is indicative of the flexibility in the flux distributions that can be achieved with a given set of metabolic reactions. The solutions to Equation 1 lie in a restricted region. This subspace defines the capabilities of
25   the *metabolic genotype* of a given organism, since the allowable solutions that satisfy Equation 1 and any constraints placed on the fluxes of the system define all the metabolic flux distributions that can be achieved with a particular set of metabolic genes.

The particular utilization of the metabolic genotype can be defined as the *metabolic phenotype* that is expressed under those particular conditions. Objectives for metabolic function can be chosen to explore the 'best' use
30   of the metabolic network within a given metabolic genotype. The solution to equation 1 can be formulated as a linear programming problem, in which the flux distribution that minimizes a particular objective if found. Mathematically, this optimization can be stated as;

$$\text{Minimize } Z \qquad\qquad \text{Equation 2}$$

35
$$\text{where } Z = \sum c_i \cdot v_i = \langle c \bullet v \rangle \qquad\qquad \text{Equation 3}$$

where Z is the objective which is represented as a linear combination of metabolic fluxes v_i. The optimization can also be stated as the equivalent maximization problem; i.e. by changing the sign on Z.

This general representation of Z enables the formulation of a number of diverse objectives. These objectives can be design objectives for a strain, exploitation of the metabolic capabilities of a genotype, or physiologically
5   meaningful objective functions, such as maximum cellular growth. For this application, growth is to be defined in terms of biosynthetic requirements based on literature values of biomass composition or experimentally determined values such as those obtained from state 60. Thus, we can define biomass generation as an additional reaction flux draining intermediate metabolites in the appropriate ratios and represented as an objective function Z. In addition to draining intermediate metabolites this reaction flux can be formed to utilize energy molecules such as ATP, NADH and
10   NADPH so as to incorporate any maintenance requirement that must be met. This new reaction flux then becomes another constraint/balance equation that the system must satisfy as the objective function. It is analogous to adding an addition column to the stoichiometric matrix of Equation 1 to represent such a flux to describe the production demands placed on the metabolic system. Setting this new flux as the objective function and asking the system to maximize the value of this flux for a given set of constraints on all the other fluxes is then a method to simulate the
15   growth of the organism.

Using linear programming, additional constraints can be placed on the value of any of the fluxes in the metabolic network.

$$\beta_i \leq v_i \leq \alpha_i \qquad\qquad\qquad \textbf{Equation 4}$$

These constraints could be representative of a maximum allowable flux through a given reaction, possibly
20   resulting from a limited amount of an enzyme present in which case the value for α_i would take on a finite value. These constraints could also be used to include the knowledge of the minimum flux through a certain metabolic reaction in which case the value for β_i would take on a finite value. Additionally, if one chooses to leave certain reversible reactions or transport fluxes to operate in a forward and reverse manner the flux may remain unconstrained by setting β_i to negative infinity and α_i to positive infinity. If reactions proceed only in the forward reaction β_i is set
25   to zero while α_i is set to positive infinity. As an example, to simulate the event of a genetic deletion the flux through all of the corresponding metabolic reactions related to the gene in question are reduced to zero by setting β_i and α_i to be zero in Equation 4. Based on the *in vivo* environment where the bacteria lives one can determine the metabolic resources available to the cell for biosynthesis of essentially molecules for biomass. Allowing the corresponding transport fluxes to be active provides the *in silico* bacteria with inputs and ouputs for substrates and by-products
30   produces by the metabolic network. Therefore as an example, if one wished to simulate the absence of a particular growth substrate one simply constrains the corresponding transport fluxes allowing the metabolite to enter the cell to be zero by allowing β_i and α_i to be zero in Equation 4. On the other hand if a substrate is only allowed to enter or exit the cell via transport mechanisms, the corresponding fluxes can be properly constrained to reflect this scenario.

Together the linear programming representation of the genome-specific stoichiometric matrix as in Equation 1
35   along with any general constraints placed on the fluxes in the system, and any of the possible objective functions

completes the formulation of the *in silico* bacterial strain. The *in silico* strain can then be used to study theoretical metabolic capabilities by simulating any number of conditions and generating flux distributions through the use of linear programming. The process 50 of formulating the *in silico* strain and simulating its behavior using linear programming techniques terminates at an end state 66.

5   Thus, by adding or removing constraints on various fluxes in the network it is possible to (1) simulate a genetic deletion event and (2) simulate or accurately provide the network with the metabolic resources present in its *in vivo* environment. Using flux balance analysis it is possible to determine the affects of the removal or addition of particular genes and their associated reactions to the composition of the metabolic genotype on the range of possible metabolic phenotypes. If the removal/deletion does not allow the metabolic network to produce necessary precursors

10   for growth, and the cell can not obtain these precursors from its environment, the deletion(s) has the potential as an antimicrobial drug target. Thus by adjusting the constraints and defining the objective function we can explore the capabilities of the metabolic genotype using linear programming to optimize the flux distribution through the metabolic network. This creates what we will refer to as an *in silico* bacterial strain capable of being studied and manipulated to analyze, interpret, and predict the genotype-phenotype relationship. It can be applied to assess the affects of

15   incremental changes in the genotype or changing environmental conditions, and provide a tool for computer aided experimental design. It should be realized that other types of organisms can similarly be represented *in silico* and still be within the scope of the invention.

The construction of a genome specific stoichiometric matrix and *in silico* microbial strains can also be applied to the area of signal transduction. The components of signaling networks can be identified within a genome and used

20   to construct a content matrix that can be further analyzed using various techniques to be determined in the future.

Example 1: E. coli metabolic genotype and in silico model

Using the methods disclosed in Figures 1 and 2, an *in silico* strain of *Escherichia coli* K-12 has been constructed and represents the first such strain of a bacteria largely generated from annotated sequence data and from biochemical information. The genetic sequence and open reading frame identifications and assignments are

25   readily available from a number of on-line locations (ex: www.tigr.org). For this example we obtained the annotated sequence from the following website for the *E. coli* Genome Project at the University of Wisconsin (http://www.genetics.wisc.edu/). Details regarding the actual sequencing and annotation of the sequence can be found at that site. From the genome annotation data the subset of genes involved in cellular metabolism was determined as described above in Figure 1, state 20, comprising the metabolic genotype of the particular strain of *E. coli*.

30   Through detailed analysis of the published biochemical literature on *E. coli* we determined (1) all of the reactions associated with the genes in the metabolic genotype and (2) any additional reactions known to occur from biochemical data which were not represented by the genes in the metabolic genotype. This provided all of the necessary information to construct the genome specific stoichiometric matrix for *E. coli* K-12.

Briefly, the *E. coli* K-12 bacterial metabolic genotype and more specifically the genome specific

35   stoichiometric matrix contains 731 metabolic processes that influence 436 metabolites (dimensions of the genome specific stoichiometric matrix are 436 x 731). There are 80 reactions present in the genome specific stoichiometric

matrix that do not have a genetic assignment in the annotated genome, but are known to be present from biochemical data. The genes contained within this metabolic genotype are shown in Table 1 along with the corresponding reactions they carry out.

5   Because *E. coli* is arguably the best studied organism, it was possible to determine the uptake rates and maintenance requirements (state 62 of Figure 2) by reference to the published literature. This *in silico* strain accounts for the metabolic capabilities of *E. coli*. It includes membrane transport processes, the central catabolic pathways, utilization of alternative carbon sources and the biosynthetic pathways that generate all the components of the biomass. In the case of *E. coli* K-12, we can call upon the wealth of data on overall metabolic behavior and detailed biochemical information about the *in vivo* genotype to which we can compare the behavior of the *in silico* strain. One

10   utility of FBA is the ability to learn about the physiology of the particular organism and explore its metabolic capabilities without any specific biochemical data. This ability is important considering possible future scenarios in which the only data that we may have for a newly discovered bacterium (perhaps pathogenic) could be its genome sequence.

Example 2: in silico deletion analysis for E. coli to find antimicrobial targets

Using the *in silico* strain constructed in Example 1, the effect of individual deletions of all the enzymes in central metabolism can be examined *in silico*. For the analysis to determine sensitive linkages in the metabolic network of *E. coli*, the objective function utilized is the maximization of the biomass yield. This is defined as a flux draining the necessary biosynthetic precursors in the appropriate ratios. This flux is defined as the biomass composition, which can be determined from the literature. See Neidhardt et. al., *Escherichia coli and Salmonella: Cellular and Molecular Biology*, Second Edition, ASM Press, Washington D.C., 1996. Thus, the objective function is the maximization of a single flux, this biosynthetic flux.

Constraints are placed on the network to account for the availability of substrates for the growth of *E. coli*. In the initial deletion analysis, growth was simulated in an aerobic glucose minimal media culture. Therefore, the constraints are set to allow for the components included in the media to be taken up. The specific uptake rate can be included if the value is known, otherwise, an unlimited supply can be provided. The uptake rate of glucose and oxygen have been determined for *E. coli* (Neidhardt et. al., *Escherichia coli and Salmonella: Cellular and Molecular Biology*, Second Edition, ASM Press, Washington D.C., 1996. Therefore, these values are included in the analysis. The uptake rate for phosphate, sulfur, and nitrogen source is not precisely known, so constraints on the fluxes for the uptake of these important substrates is not included, and the metabolic network is allowed to take up any required amount of these substrates.

The results showed that a high degree of redundancy exists in central intermediary metabolism during growth in glucose minimal media, which is related to the interconnectivity of the metabolic reactions. Only a few metabolic functions were found to be essential such that their loss removes the capability of cellular growth on glucose. For growth on glucose, the essential gene products are involved in the 3-carbon stage of glycolysis, three reactions of the

TCA cycle, and several points within the PPP. Deletions in the 6-carbon stage of glycolysis result in a reduced ability to support growth due to the diversion of additional flux through the PPP.

The results from the gene deletion study can be directly compared with growth data from mutants. The growth characteristics of a series of *E. coli* mutants on several different carbon sources were examined (80 cases were determined from the literature), and compared to the *in silico* deletion results (Table 2). The majority (73 of 80 cases or 91%) of the mutant experimental observations are consistent with the predictions of the *in silico* study. The results from the *in silico* gene deletion analysis are thus consistent with experimental observations.

Example 3: Prediction of genome scale shifts in gene expression

Flux based analysis can be used to predict metabolic phenotypes under different growth conditions, such as substrate and oxygen availability. The relation between the flux value and the gene expression levels is non-linear, resulting in bifurcations and multiple steady states. However, FBA can give qualitative (on/off) information as well as
5   the relative importance of gene products under a given condition. Based on the magnitude of the metabolic fluxes, qualitative assessment of gene expression can be inferred.

Figure 3a shows the five phases of distinct metabolic behavior of *E. Coli* in response to varying oxygen availability, going from completely anaerobic (phase I) to completely aerobic (phase V). Figures 3b and 3c display lists of the genes that are predicted to be induced or repressed upon the shift from aerobic growth (phase V) to nearly
10 complete anaerobic growth (phase II). The numerical values shown in Figures 3b and 3c are the fold change in the magnitude of the fluxes calculated for each of the listed enzymes.

For this example, the objective of maximization of biomass yield is utilized (as described above). The constraints on the system are also set accordingly (as described above). However, in this example, a change in the availability of a key substrate is leading to changes in the metabolic behavior. The change in the parameter is reflected
15 as a change in the uptake flux. Therefore, the maximal allowable oxygen uptake rate is changed to generate this data. The figure demonstrates how several fluxes in the metabolic network will change as the oxygen uptake flux is continuously decreased. Therefore, the constraints on the fluxes is identical to what is described in the previous section, however, the oxygen uptake rate is set to coincide with the point in the diagram.

Corresponding experimental data sets are now becoming available. Using high-density oligonucleotide arrays
20 the expression levels of nearly every gene in *Saccharomyces cerevisiae* can now be analyzed under various growth conditions. From these studies it was shown that nearly 90% of all yeast mRNAs are present in growth on rich and minimal media, while a large number of mRNAs were shown to be differentially expressed under these two conditions. Another recent article shows how the metabolic and genetic control of gene expression can be studied on a genomic scale using DNA microarray technology (*Exploring the Metabolic and Genetic Control of Gene Expression on a Genomic*
25 *Scale*, Science, Vol. 278, October 24, 1997. The temporal changes in genetic expression profiles that occur during the diauxic shift in *S. cerevisiae* were observed for every known expressed sequence tag (EST) in this genome. As shown above, FBA can be used to qualitatively simulate shifts in metabolic genotype expression patterns due to alterations in

growth environments. Thus, FBA can serve to complement current studies in metabolic gene expression, by providing a fundamental approach to analyze, interpret, and predict the data from such experiments.

Example 4: Design of defined media

An important economic consideration in large-scale bioprocesses is optimal medium formulation. FBA can be
5   used to design such media. Following the approach defined above, a flux-balance model for the first completely sequenced free living organism, *Haemophilus influenzae*, has been generated. One application of this model is to predict a minimal defined media. It was found that *H. influenzae* can grow on the minimal defined medium as determined from the ORF assignments and predicted using FBA. Simulated bacterial growth was predicted using the following defined media: fructose, arginine, cysteine, glutamate, putrescine, spermidine, thiamin, NAD, tetrapyrrole,
10   pantothenate, ammonia, phosphate. This predicted minimal medium was compared to the previously published defined media and was found to differ in only one compound, inosine. It is known that inosine is not required for growth, however it does serve to enhance growth. Again the *in silico* results obtained were consistent with published *in vivo* research. These results provide confidence in the use of this type of approach for the design of defined media for organisms in which there currently does not exist a defined media.

15   While particular embodiments of the invention have been described in detail, it will be apparent to those skilled in the art that these embodiments are exemplary rather than limiting, and the true scope of the invention is defined by the claims that follow.

Table 1

The genes included in the E. coli metabolic genotype along with corresponding enzymes and reactions that comprise the genome specific stoichiometric matrix. The final column indicates the presence/absence of the gene (as the number of copies) in the E. coli genome. Thus the presence of a gene in the E. coli genome indicates that the gene is part of the metabolic genotype. Reactions/Genes not present in the genome are those gathered at state 56 in Figure 2 and together with the reactions of the genes in the metabolic genotype form the columns of the genome specific stoichiometric matrix.

Enzyme	Gene	Reaction	E. coli genome
Glucokinase	glk	GLC + ATP -> G6P + ADP	1
Glucokinase	glk	bDGLC + ATP -> bDG6P + ADP	1
Phosphoglucose isomerase	pgi	G6P <-> F6P	1
Phosphoglucose isomerase	pgi	bDG6P <-> G6P	1
Phosphoglucose isomerase	pgi	bDG6P <-> F6P	1
Aldose 1-epimerase	galM	bDGLC <-> GLC	1
Glucose-1-phophatase	agp	G1P -> GLC + PI	1
Phosphofructokinase	pfkA	F6P + ATP -> FDP + ADP	1
Phosphofructokinase B	pfkB	F6P + ATP -> FDP + ADP	1
Fructose-1,6-bisphosphatase	fbp	FDP -> F6P + PI	1
Fructose-1,6-bisphosphatate aldolase	fba	FDP <-> T3P1 + T3P2	2
Triosphosphate Isomerase	tpiA	T3P1 <-> T3P2	1
Methylglyoxal synthase	mgsA	T3P2 -> MTHGXL + PI	0
Glyceraldehyde-3-phosphate dehydrogenase-A complex	gapA	T3P1 + PI + NAD <-> NADH + 13PDG	1
Glyceraldehyde-3-phosphate dehydrogenase-C complex	gapC1C2	T3P1 + PI + NAD <-> NADH + 13PDG	2

Enzyme	Gene	Reaction	E. coli genome
Phosphoglycerate kinase	pgk	13PDG + ADP <-> 3PG + ATP	1
Phosphoglycerate mutase 1	gpmA	3PG <-> 2PG	1
Phosphoglycerate mutase 2	gpmB	3PG <-> 2PG	1
Enolase	eno	2PG <-> PEP	1
Phosphoenolpyruvate synthase	ppsA	PYR + ATP -> PEP + AMP + PI	1
Pyruvate Kinase II	pykA	PEP + ADP -> PYR + ATP	1
Pyruvate Kinase I	pykF	PEP + ADP -> PYR + ATP	1
Pyruvate dehydrogenase	lpdA, aceEF	PYR + COA + NAD -> NADH + CO2 + ACCOA	3
Glucose-1-phosphate adenylyltransferase	glgC	ATP + G1P -> ADPGLC + PPI	1
Glycogen synthase	glgA	ADPGLC -> ADP + GLYCOGEN	1
Glycogen phosphorylase	glgP	GLYCOGEN + PI -> G1P	1
Maltodextrin phosphorylase	malP	GLYCOGEN + PI -> G1P	1
Glucose 6-phosphate-1-dehydrogenase	zwf	G6P + NADP <-> D6PGL + NADPH	1
6-Phosphogluconolactonase	pgl	D6PGL -> D6PGC	0
6-Phosphogluconate dehydrogenase (decarboxylating)	gnd	D6PGC + NADP -> NADPH + CO2 + RL5P	1
Ribose-5-phosphate isomerase A	rpiA	RL5P <-> R5P	1
Ribose-5-phosphate isomerase B	rpiB	RL5P <-> R5P	1
Ribulose phosphate 3-epimerase	rpe	RL5P <-> X5P	1
Transketolase I	tktA	R5P + X5P <-> T3P1 + S7P	1
Transketolase II	tktB	R5P + X5P <-> T3P1 + S7P	1
Transketolase I	tktA	X5P + E4P <-> F6P + T3P1	1

Enzyme	Gene	Reaction	E. coli genome
Transketolase II	tktB	X5P + E4P <-> F6P + T3P1	1
Transaldolase B	talB	T3P1 + S7P <-> E4P + F6P	1
Phosphogluconate dehydratase	edd	D6PGC -> 2KD6PG	1
2-Keto-3-deoxy-6-phosphogluconate aldolase	eda	2KD6PG -> T3P1 + PYR	1
Citrate synthase	gltA	ACCOA + OA -> COA + CIT	1
Aconitase A	acnA	CIT <-> ICIT	1
Aconitase B	acnB	CIT <-> ICIT	1
Isocitrate dehydrogenase	icdA	ICIT + NADP <-> CO2 + NADPH + AKG	1
2-Ketoglutarate dehydrogenase	sucAB, lpdA	AKG + NAD + COA -> CO2 + NADH + SUCCOA	3
Succinyl-CoA synthetase	sucCD	SUCCOA + ADP + PI <-> ATP + COA + SUCC	2
Succinate dehydrogenase	sdhABCD	SUCC + FAD -> FADH + FUM	4
Fumarate reductase	frdABCD	FUM + FADH -> SUCC + FAD	4
Fumarase A	fumA	FUM <-> MAL	1
Fumarase B	fumB	FUM <-> MAL	1
Fumarase C	fumC	FUM <-> MAL	1
Malate dehydrogenase	mdh	MAL + NAD <-> NADH + OA	1
D-Lactate dehydrogenase 1	dld	PYR + NADH <-> NAD + LAC	1
D-Lactate dehydrogenase 2	lldA	PYR + NADH <-> NAD + LAC	1
Acetaldehyde dehydrogenase	adhE	ACCOA + 2 NADH <-> ETH + 2 NAD + COA	1
Pyruvate formate lyase 1	pflAB	PYR + COA -> ACCOA + FOR	2
Pyruvate formate lyase 2	pflCD	PYR + COA -> ACCOA + FOR	2

Enzyme	Gene	Reaction	E. coli genome
Formate hydrogen lyase	fdhF, hycBEFG	FOR -> CO2	5
Phosphotransacetylase	pta	ACCOA + PI <-> ACTP + COA	1
Acetate kinase A	ackA	ACTP + ADP <-> ATP + AC	1
GAR transformylase T	purT	ACTP + ADP <-> ATP + AC	1
Acetyl-CoA synthetase	acs	ATP + AC + COA -> AMP + PPI + ACCOA	1
Phosphoenolpyruvate carboxykinase	pckA	OA + ATP -> PEP + CO2 + ADP	1
Phosphoenolpyruvate carboxylase	ppc	PEP + CO2 -> OA + PI	1
Malic enzyme (NADP)	maeB	MAL + NADP -> CO2 + NADPH + PYR	0
Malic enzyme (NAD)	sfcA	MAL + NAD -> CO2 + NADH + PYR	1
Isocitrate lyase	aceA	ICIT -> GLX + SUCC	1
Malate synthase A	aceB	ACCOA + GLX -> COA + MAL	1
Malate synthase G	glcB	ACCOA + GLX -> COA + MAL	1
Inorganic pyrophosphatase	ppa	PPI -> 2 PI	1
NADH dehydrogenase II	ndh	NADH + Q -> NAD + QH2	1
NADH dehydrogenase I	nuoABEFGHIJKLMN	NADH + Q -> NAD + QH2 +3.5 HEXT	1
Formate dehydrogenase-N	fdnGHI	FOR + Q -> QH2 + CO2 +2 HEXT	3
Formate dehydrogenase-O	fdoHG	FOR + Q -> QH2 + CO2 +2 HEXT	3
Formate dehydrogenase	fdhF	FOR + Q -> QH2 + CO2 +2 HEXT	1
Pyruvate oxidase	poxB	PYR + Q -> AC + CO2 + QH2	1
Glycerol-3-phosphate dehydrogenase (aerobic)	glpD	GL3P + Q -> T3P2 + QH2	1
Glycerol-3-phosphate dehydrogenase (anaerobic)	glpABC	GL3P + Q -> T3P2 + QH2	3

Enzyme	Gene	Reaction	E. coli genome
Cytochrome oxidase bo3	cyoABCD, cycBC, cydAB	QH2 + .5 O2 -> Q + 2.5 HEXT	6
Cytochrome oxidase bd	cydABCD, appBC, cycBC	QH2 + .5 O2 -> Q + 2 HEXT	6
Succinate dehydrogenase complex	sdhABCD	FADH + Q <-> FAD + QH2	4
Thioredoxin reductase	trxB	QTHIO + NADPH -> NADP + RTHIO	1
Pyridine nucleotide transhydrogenase	pntAB	NADPH + NAD -> NADP + NADH	2
Pyridine nucleotide transhydrogenase	pntAB	NADP + NADH + 2 HEXT -> NADPH + NAD	2
Hydrogenase I	hyaABC	2 Q + 2 HEXT <-> 2 QH2 + H2	3
Hydrogenase 2	hybAC	2 Q + 2 HEXT <-> 2 QH2 + H2	2
Hydrogenase 3	hycFGBE	2 Q + 2 HEXT <-> 2 QH2 + H2	4
F0F1-ATPase	atpABCDEFGHI	ATP <-> ADP + PI + 4 HEXT	9
Alpha-galactosidase (melibiase)	melA	MELI -> GLC + GLAC	1
Galactokinase	galK	GLAC + ATP -> GAL1P + ADP	1
Galactose-1-phosphate uridylyltransferase	galT	GAL1P + UDPG <-> G1P + UDPGAL	1
UDP-glucose 4-epimerase	galE	UDPGAL <-> UDPG	1
UDP-glucose-1-phosphate uridylyltransferase	galU	G1P + UTP <-> UDPG + PPI	1
Phosphoglucomutase	pgm	G1P <-> G6P	1
Periplasmic beta-glucosidase precursor	bglX	LCTS -> GLC + GLAC	1
Beta-galactosidase (LACTase)	lacZ	LCTS -> GLC + GLAC	1
trehalose-6-phosphate hydrolase	treC	TRE6P -> bDG6P + GLC	1
Beta-fructofuranosidase		SUC6P -> G6P + FRU	0
1-Phosphofructokinase (Fructose 1-phosphate kinase)	fruK	F1P + ATP -> FDP + ADP	1

Enzyme	Gene	Reaction	E. coli genome
Xylose isomerase	xylA	FRU -> GLC	1
Phosphomannomutase	cpsG	MAN6P <-> MAN1P	1
Mannose-6-phosphate isomerase	manA	MAN1P <-> F6P	1
N-Acetylglucosamine-6-phosphate deacetylase	nagA	NAGP -> GA6P + AC	1
Glucosamine-6-phosphate deaminase	nagB	GA6P -> F6P + NH3	1
N-Acetylneuraminate lyase	nanA	SLA -> PYR + NAMAN	1
L-Fucose isomerase	fucI	FUC <-> FCL	1
L-Fuculokinase	fucK	FCL + ATP -> FCL1P + ADP	1
L-Fuculose phosphate aldolase	fucA	FCL1P <-> LACAL + T3P2	1
Lactaldehyde reductase	fucO	LACAL + NADH <-> 12PPD + NAD	1
Aldehyde dehydrogenase A	aldA	LACAL + NAD <-> LLAC + NADH	1
Aldehyde dehydrogenase B	aldB	LACAL + NAD <-> LLAC + NADH	1
Aldehyde dehydrogenase	adhC	LACAL + NAD <-> LLAC + NADH	1
Aldehyde dehydrogenase	adhC	GLAL + NADH <-> GL + NAD	1
Aldehyde dehydrogenase	adhE	LACAL + NAD -> LLAC + NADH	1
Aldehyde dehydrogenase	aldH	LACAL + NAD <-> LLAC + NADH	1
Aldehyde dehydrogenase	aldH	ACAL + NAD -> AC + NADH	1
Gluconokinase I	gntV	GLCN + ATP -> D6PGC + ADP	1
Gluconokinase II	gntK	GLCN + ATP -> D6PGC + ADP	1
L-Rhamnose isomerase	rhaA	RMN <-> RML	1
Rhamnulokinase	rhaB	RML + ATP -> RML1P + ADP	1

Enzyme	Gene	Reaction	E. coli genome
Rhamnulose-1-phosphate aldolase	rhaD	RML1P <-> LACAL + T3P2	1
L-Arabinose isomerase	araA	ARAB <-> RBL	1
Arabinose-5-phosphate isomerase		RL5P <-> A5P	0
L-Ribulokinase	araB	RBL + ATP -> RL5P + ADP	1
L-Ribulose-phosphate 4-epimerase	araD	RL5P <-> X5P	1
Xylose isomerase	xylA	XYL <-> XUL	1
Xylulokinase	xylB	XUL + ATP -> X5P + ADP	1
Ribokinase	rbsK	RIB + ATP -> R5P + ADP	1
Mannitol-1-phosphate 5-dehydrogenase	mtlD	MNT6P + NAD <-> F6P + NADH	1
Glucitol-6-phosphate dehydrogenase	srlD	GLT6P + NAD <-> F6P + NADH	1
Galactitol-1-phosphate dehydrogenase	gatD	GLT1P + NAD <-> TAG6P + NADH	1
Phosphofructokinase B	pfkB	TAG6P + ATP -> TAG16P + ADP	1
1-Phosphofructokinase	fruK	TAG6P + ATP -> TAG16P + ADP	1
Tagatose-6-phosphate kinase	agaZ	TAG6P + ATP -> TAG16P + ADP	1
Tagatose-bisphosphate aldolase 2	gatY	TAG16P <-> T3P2 + T3P1	1
Tagatose-bisphosphate aldolase 1	agaY	TAG16P <-> T3P2 + T3P1	1
Glycerol kinase	glpK	GL + ATP -> GL3P + ADP	1
Glycerol:3-phosphate dehydrogenase (NAD(P)+)	gpsA	GL3P + NADP <-> T3P2 + NADPH	1
Phosphopentomutase	deoB	DR1P <-> DR5P	1
Phosphopentomutase	deoB	R1P <-> R5P	1
Deoxyribose-phosphate aldolase	deoC	DR5P -> ACAL + T3P1	1

Enzyme	Gene	Reaction	E. coli genome
Asparate transaminase	aspC	OA + GLU <-> ASP + AKG	1
Asparagine synthetase (Glutamate dependent)	asnB	ASP + ATP + GLN -> GLU + ASN + AMP + PPI	1
Aspartate-ammonia ligase	asnA	ASP + ATP + NH3 -> ASN + AMP + PPI	1
Glutamate dehydrogenase	gdhA	AKG + NH3 + NADPH <-> GLU + NADP	1
Glutamate-ammonia ligase	glnA	GLU + NH3 + ATP -> GLN + ADP + PI	1
Glutamate synthase	gltBD	AKG + GLN + NADPH -> NADP + 2 GLU	2
Alanine transaminase	alaB	PYR + GLU <-> AKG + ALA	0
Valine-pyruvate aminotransferase	avtA	OIVAL + ALA -> PYR + VAL	1
Alanine racemase, biosynthetic	alr	ALA <-> DALA	1
Alanine racemase, catabolic	dadX	ALA -> DALA	1
N-Acetylglutamate synthase	argA	GLU + ACCOA -> COA + NAGLU	1
N-Acetylglutamate kinase	argB	NAGLU + ATP -> ADP + NAGLUYP	1
N-Acetylglutamate phosphate reductase	argC	NAGLUYP + NADPH <-> NADP + PI + NAGLUSAL	1
Acetylornithine transaminase	argD	NAGLUSAL + GLU <-> AKG + NAARON	1
Acetylornithine deacetylase	argE	NAARON -> AC + ORN	1
Carbamoyl phosphate synthetase	carAB	GLN +2 ATP + CO2 -> GLU + CAP +2 ADP + PI	2
Ornithine carbamoyl transferase 1	argF	ORN + CAP <-> CITR + PI	2
Ornithine carbamoyl transferase 2	argI	ORN + CAP <-> CITR + PI	1
Ornithine transaminase	ygjGH	ORN + AKG -> GLUGSAL + GLU	2
Argininosuccinate synthase	argG	CITR + ASP + ATP -> AMP + PPI + ARGSUCC	1
Argininosuccinate lyase	argH	ARGSUCC <-> FUM + ARG	1

-20-

Enzyme	Gene	Reaction	E. coli genome
Arginine decarboxylase, biosynthetic	speA	ARG -> CO2 + AGM	1
Arginine decarboxylase, degradative	adi	ARG -> CO2 + AGM	1
Agmatinase	speB	AGM -> UREA + PTRC	1
Ornithine decarboxylase, biosynthetic	speC	ORN -> PTRC + CO2	1
Ornithine decarboxylase, degradative	speF	ORN -> PTRC + CO2	1
Adenosylmethionine decarboxylase	speD	SAM <-> DSAM + CO2	1
Spermidine synthase	speE	PTRC + DSAM -> SPMD + 5MTA	1
Methylthioadenosine nucleosidase		5MTA -> AD + 5MTR	0
5-Methylthioribose kinase		5MTR + ATP -> 5MTRP + ADP	0
5-Methylthioribose-1-phosphate isomerase		5MTRP <-> 5MTR1P	0
E-1 (Enolase-phosphatase)		5MTR1P -> DKMPP	0
E-3 (Unknown)		DKMPP -> FOR + KMB	0
Transamination (Unknown)		KMB + GLN -> GLU + MET	0
γ-Glutamyl kinase	proB	GLU + ATP -> ADP + GLUP	1
Glutamate-5-semialdehyde dehydrogenase	proA	GLUP + NADPH -> NADP + PI + GLUGSAL	1
N-Acetylornithine deacetylase	argE	NAGLUSAL -> GLUGSAL + AC	1
Pyrroline-5-carboxylate reductase	proC	GLUGSAL + NADPH -> PRO + NADP	1
Threonine dehydratase, biosynthetic	ilvA	THR -> NH3 + OBUT	1
Threonine dehydratase, catabolic	tdcB	THR -> NH3 + OBUT	1
Acetohydroxybutanoate synthase I	ilvBN	OBUT + PYR -> ABUT + CO2	2
Acetohydroxybutanoate synthase II	ilvGI(12)M	OBUT + PYR -> ABUT + CO2	3

Enzyme	Gene	Reaction	E. coli genome
Acetohydroxybutanoate synthase III	ilvH	OBUT + PYR · -> ABUT + CO2	2
Acetohydroxy Acid isomeroreductase	ilvC	ABUT + NADPH · -> NADP + DHMVA	1
Dihydroxy acid dehydratase	ilvD	DHMVA · -> OMVAL	1
Branched chain amino acid aminotransferase	ilvE	OMVAL + GLU · <-> AKG + ILE	1
Acetolactate synthase I	ilvBN	2 PYR · -> CO2 + ACLAC	2
Acetolactate synthase II	ilvG(12)M	2 PYR · -> CO2 + ACLAC	3
Acetolactate synthase III	ilvIH	2 PYR · -> CO2 + ACLAC	2
Acetohydroxy acid isomeroreductase	ilvC	ACLAC + NADPH · -> NADP + DHVAL	1
Dihydroxy acid dehydratase	ilvD	DHVAL · -> OIVAL	1
Branched chain amino acid aminotransferase	ilvE	OIVAL + GLU · -> AKG + VAL	1
Valine-pyruvate aminotransferase	avtA	OIVAL + ALA · -> PYR + VAL	1
Isopropylmalate synthase	leuA	ACCOA + OIVAL · -> COA + CBHCAP	1
Isopropylmalate isomerase	leuCD	CBHCAP · <-> IPPMAL	2
3-Isopropylmalate dehydrogenase	leuB	IPPMAL + NAD · -> NADH + OICAP + CO2	1
Branched chain amino acid aminotransferase	ilvE	OICAP + GLU · -> AKG + LEU	1
Aromatic amino acid transaminase	tyrB	OICAP + GLU · -> AKG + LEU	1
2-Dehydro-3-deoxyphosphoheptonate aldolase F	aroF	E4P + PEP · -> PI + 3DDAH7P	1
2-Dehydro-3-deoxyphosphoheptonate aldolase G	aroG	E4P + PEP · -> PI + 3DDAH7P	1
2-Dehydro-3-deoxyphosphoheptonate aldolase H	aroH	E4P + PEP · -> PI + 3DDAH7P	1
3-Dehydroquinate synthase	aroB	3DDAH7P · -> DQT + PI	1
3-Dehydroquinate dehydratase	aroD	DQT · <-> DHSK	1

Enzyme	Gene	Reaction	E. coli genome
Shikimate dehydrogenase	aroE	DHSK + NADPH <-> SME + NADP	1
Shikimate kinase I	aroK	SME + ATP -> ADP + SME5P	1
Shikimate kinase II	aroL	SME + ATP -> ADP + SME5P	1
3-Phosphoshikimate-1-carboxyvinyltransferase	aroA	SME5P + PEP <-> 3PSME + PI	1
Chorismate synthase	aroC	3PSME -> PI + CHOR	1
Chorismate mutase 1	pheA	CHOR -> PHEN	1
Prephenate dehydratase	pheA	PHEN -> CO2 + PHPYR	1
Aromatic amino acid transaminase	tyrB	PHPYR + GLU <-> AKG + PHE	1
Chorismate mutase 2	tyrA	CHOR -> PHEN	1
Prephenate dehydrogenase	tyrA	PHEN + NAD -> HPHPYR + CO2 + NADH	1
Aromatic amino acid transaminase	tyrB	HPHPYR + GLU <-> AKG + TYR	1
Asparate transaminase	aspC	HPHPYR + GLU <-> AKG + TYR	1
Anthranilate synthase	trpDE	CHOR + GLN -> GLU + PYR + AN	2
Anthranilate synthase component II	trpD	AN + PRPP -> PPI + NPRAN	1
Phosphoribosyl anthranilate isomerase	trpC	NPRAN -> CPAD5P	1
Indoleglycerol phosphate synthase	trpC	CPAD5P -> CO2 + IGP	1
Tryptophan synthase	trpAB	IGP + SER -> T3P1 + TRP	2
Phosphoribosyl pyrophosphate synthase	prsA	R5P + ATP <-> PRPP + AMP	1
ATP phosphoribosyltransferase	hisG	PRPP + ATP -> PPI + PRBATP	1
Phosphoribosyl-ATP pyrophosphatase	hisIE	PRBATP -> PPI + PRBAMP	1
Phosphoribosyl-AMP cyclohydrolase	hisIE	PRBAMP -> PRFP	1

Enzyme	Gene	Reaction	E. coli genome
Phosphoribosylformimino-5-amino-1-phosphoribosyl-4-imidazole carboxamide isomerase	hisA	PRFP -> PRLP	1
Imidazoleglycerol phosphate synthase	hisFH	PRLP + GLN -> GLU + AICAR + DIMGP	2
Imidazoleglycerol phosphate dehydratase	hisB	DIMGP -> IMACP	1
L-Histidinol phosphate aminotransferase	hisC	IMACP + GLU -> AKG + HISOLP	1
Histidinol phosphatase	hisB	HISOLP -> PI + HISOL	1
Histidinol dehydrogenase	hisD	HISOL + 3 NAD -> HIS + 3 NADH	1
3-Phosphoglycerate dehydrogenase	serA	3PG + NAD -> NADH + PHP	1
Phosphoserine transaminase	serC	PHP + GLU -> AKG + 3PSER	1
Phosphoserine phosphatase	serB	3PSER -> PI + SER	1
Glycine hydroxymethyltransferase	glyA	THF + SER -> GLY + METTHF	1
Threonine dehydrogenase	tdh	THR + COA -> GLY + ACCOA	1
Amino ketobutyrate CoA ligase	kbl	THR + COA -> GLY + ACCOA	1
Sulfate adenylyltransferase	cysDN	SLF + ATP + GTP -> PPI + APS + GDP + PI	2
Adenylylsulfate kinase	cysC	APS + ATP -> ADP + PAPS	1
3'-Phospho-adenylylsulfate reductase	cysH	PAPS + RTHIO -> OTHIO + H2SO3 + PAP	1
Sulfite reductase	cysIJ	H2SO3 + 3 NADPH <-> H2S + 3 NADP	2
Serine transacetylase	cysE	SER + ACCOA <-> COA + ASER	1
O-Acetylserine (thiol)-lyase A	cysK	ASER + H2S -> AC + CYS	1
O-Acetylserine (thiol)-lyase B	cysM	ASER + H2S -> AC + CYS	1
3'-5' Bisphosphate nucleotidase		PAP -> AMP + PI	0

-24-

Enzyme	Gene	Reaction	E. coli genome
Aspartate kinase I	thrA	ASP + ATP <-> ADP + BASP	1
Aspartate kinase II	metL	ASP + ATP <-> ADP + BASP	1
Aspartate kinase III	lysC	ASP + ATP <-> ADP + BASP	1
Aspartate semialdehyde dehydrogenase	asd	BASP + NADPH <-> NADP + PI + ASPSA	1
Homoserine dehydrogenase I	thrA	ASPSA + NADPH <-> NADP + HSER	1
Homoserine dehydrogenase II	metL	ASPSA + NADPH <-> NADP + HSER	1
Homoserine kinase	thrB	HSER + ATP -> ADP + PHSER	1
Threonine synthase	thrC	PHSER -> PI + THR	1
Dihydrodipicolinate synthase	dapA	ASPSA + PYR -> D23PIC	1
Dihydrodipicolinate reductase	dapB	D23PIC + NADPH -> NADP + PIP26DX	1
Tetrahydrodipicolinate succinylase	dapD	PIP26DX + SUCCOA -> COA + NS2A6O	1
Succinyl diaminopimelate aminotransferase	dapC	NS2A6O + GLU <-> AKG + NS26DP	0
Succinyl diaminopimelate desuccinylase	dapE	NS26DP -> SUCC + D26PIM	1
Diaminopimelate epimerase	dapF	D26PIM <-> MDAP	1
Diaminopimelate decarboxylase	lysA	MDAP -> CO2 + LYS	1
Lysine decarboxylase 1	cadA	LYS -> CO2 + CADV	1
Lysine decarboxylase 2	ldcC	LYS -> CO2 + CADV	1
Homoserine transsuccinylase	metA	HSER + SUCCOA -> COA + OSLHSER	1
O-succinlyhomoserine lyase	metB	OSLHSER + CYS -> SUCC + LLCT	1
Cystathionine-β-lyase	metC	LLCT -> HCYS + PYR + NH3	1
Adenosyl homocysteinase (Unknown)	Unknown	HCYS + ADN <-> SAH	0

Enzyme	Gene	Reaction	E. coli genome
Cobalamin-dependent methionine synthase	metH	HCYS + MTHF -> MET + THF	1
Cobalamin-independent methionine synthetase	metE	HCYS + MTHF -> MET + THF	1
S-Adenosylmethionine synthetase	metK	MET + ATP -> PPI + PI + SAM	1
D-Amino acid dehydrogenase	dadA	DALA + FAD -> FADH + PYR + NH3	0
Putrescine transaminase	pat	PTRC + AKG -> GABAL + GLU	1
Amino oxidase	tynA	PTRC -> GABAL + NH3	0
Aminobutyraldehyde dehydrogenase	prr	GABAL + NAD -> GABA + NADH	0
Aldehyde dehydrogenase	aldH	GABAL + NAD -> GABA + NADH	1
Aminobutyrate aminotransaminase 1	gabT	GABA + AKG -> SUCCSAL + GLU	1
Aminobutyrate aminotransaminase 2	goaG	GABA + AKG -> SUCCSAL + GLU	1
Succinate semialdehyde dehydrogenase –NAD	sad	SUCCSAL + NAD -> SUCC + NADH	0
Succinate semialdehyde dehydrogenase –NADP	gabD	SUCCSAL + NADP -> SUCC + NADPH	1
Asparininase I	ansA	ASN -> ASP + NH3	1
Asparininase II	ansB	ASN -> ASP + NH3	1
Aspartate ammonia-lyase	aspA	ASP -> FUM + NH3	1
Tryptophanase	tnaA	CYS -> PYR + NH3 + H2S	1
L-Cysteine desulfhydrase		CYS -> PYR + NH3 + H2S	0
Glutamate decarboxylase A	gadA	GLU -> GABA + CO2	1
Glutamate decarboxylase B	gadB	GLU -> GABA + CO2	1
Glutaminase A		GLN -> GLU + NH3	0
Glutaminase B		GLN -> GLU + NH3	0

Enzyme	Gene	Reaction	E. coli genome
Proline dehydrogenase	putA	PRO + FAD -> FADH + GLUGSAL	1
Pyrroline-5-carboxylate dehydrogenase	putA	GLUGSAL + NAD -> NADH + GLU	1
Serine deaminase 1	sdaA	SER -> PYR + NH3	1
Serine deaminase 2	sdaB	SER -> PYR + NH3	1
Tryptophanase	tnaA	SER -> PYR + NH3	1
D-Serine deaminase	dsdA	DSER -> PYR + NH3	1
Threonine dehydrogenase	tdh	THR + NAD -> 2A3O + NADH	1
Amino ketobutyrate ligase	kbl	2A3O + COA -> ACCOA + GLY	1
Threonine dehydratase catabolic	tdcB	THR -> OBUT + NH3	1
Threonine deaminase 1	sdaA	THR -> OBUT + NH3	1
Threonine deaminase 2	sdaB	THR -> OBUT + NH3	1
Tryptophanase	tnaA	TRP <-> INDOLE + PYR + NH3	1
Amidophoshoribosyl transferase	purF	PRPP + GLN -> PPI + GLU + PRAM	1
Phosphoribosylamine-glycine ligase	purD	PRAM + ATP + GLY <-> ADP + PI + GAR	1
Phosphoribosylglycinamide formyltransferase	purN	GAR + FTHF -> THF + FGAR	1
GAR transformylase T	purT	GAR + FOR + ATP -> ADP + PI + FGAR	1
Phosphoribosylformylglycinamide synthetase	purL	FGAR + ATP + GLN -> GLU + ADP + PI + FGAM	1
Phosphoribosylformylglycinamide cyclo-ligase	purM	FGAM + ATP -> ADP + PI + AIR	1
Phosphoribosylaminoimidazole carboxylase 1	purK	AIR + CO2 + ATP <-> NCAIR + ADP + PI	1
Phosphoribosylaminoimidazole carboxylase 2	purE	NCAIR <-> CAIR	1
Phosphoribosylaminoimidazole-succinocarboxamide synthetase	purC	CAIR + ATP + ASP <-> ADP + PI + SAICAR	1

Enzyme	Gene	Reaction	E. coli genome
5'-Phosphoribosyl-4-(N-succinocarboxamide)-5-aminoimidazole lyase	purB	SAICAR <-> FUM + AICAR	1
AICAR transformylase	purH	AICAR + FTHF <-> THF + PRFICA	1
IMP cyclohydrolase	purH	PRFICA <-> IMP	1
Adenylosuccinate synthetase	purA	IMP + GTP + ASP -> GDP + PI + ASUC	1
Adenylosuccinate lyase	purB	ASUC <-> FUM + AMP	1
IMP dehydrogenase	guaB	IMP + NAD -> NADH + XMP	1
GMP synthase	guaA	XMP + ATP + GLN -> GLU + AMP + PPI + GMP	1
GMP reductase	guaC	GMP + NADPH -> NADP + IMP + NH3	1
Aspartate-carbamoyltransferase	pyrBI	CAP + ASP -> CAASP + PI	2
Dihydroorotase	pyrC	CAASP <-> DOROA	1
Dihydroorotate dehydrogenase	pyrD	DOROA + Q <-> QH2 + OROA	1
Orotate phosphoribosyl transferase	pyrE	OROA + PRPP <-> PPI + OMP	1
OMP decarboxylase	pyrF	OMP -> CO2 + UMP	1
CTP synthetase	pyrG	UTP + GLN + ATP -> GLU + CTP + ADP + PI	1
Adenylate kinase	adk	ATP + AMP <-> 2 ADP	1
Adenylate kinase	adk	GTP + AMP <-> ADP + GDP	1
Adenylate kinase	adk	ITP + AMP <-> ADP + IDP	1
Adenylate kinase	adk	DAMP + ATP <-> ADP + DADP	1
Guanylate kinase	gmk	GMP + ATP <-> GDP + ADP	1
Deoxyguanylate kinase	gmk	DGMP + ATP <-> DGDP + ADP	1

Enzyme	Gene	Reaction	E. coli genome
Nucleoside-diphosphate kinase	ndk	GDP + ATP <-> GTP + ADP	1
Nucleoside-diphosphate kinase	ndk	UDP + ATP <-> UTP + ADP	1
Nucleoside-diphosphate kinase	ndk	CDP + ATP <-> CTP + ADP	1
Nucleoside-diphosphate kinase	ndk	DGDP + ATP <-> DGTP + ADP	1
Nucleoside-diphosphate kinase	ndk	DUDP + ATP <-> DUTP + ADP	1
Nucleoside-diphosphate kinase	ndk	DCDP + ATP <-> DCTP + ADP	1
Nucleoside-diphosphate kinase	ndk	DADP + ATP <-> DATP + ADP	1
Nucleoside-diphosphate kinase	ndk	DTDP + ATP <-> DTTP + ADP	1
AMP Nucleosidse	amn	AMP -> AD + R5P	1
Adenosine deaminase	add	ADN -> INS + NH3	1
Deoxyadenosine deaminase	add	DA -> DIN + NH3	1
Adenine deaminase	yicP	AD -> NH3 + HYXN	1
Inosine kinase	gsk	INS + ATP -> IMP + ADP	1
Guanosine kinase	gsk	GSN + ATP -> GMP + ADP	1
Adenosine kinase	adk	ADN + ATP -> AMP + ADP	1
Adenine phosphoryltransferase	apt	AD + PRPP -> PPI + AMP	1
Xanthine-guanine phosphoribosyltransferase	gpt	XAN + PRPP -> XMP + PPI	1
Xanthine-guanine phosphoribosyltransferase	gpt	HYXN + PRPP -> PPI + IMP	1
Hypoxanthine phosphoribosyltransferase	hpt	HYXN + PRPP -> PPI + IMP	1
Xanthine-guanine phosphoribosyltransferase	gpt	GN + PRPP -> PPI + GMP	1
Hypoxanthine phosphoribosyltransferase	hpt	GN + PRPP -> PPI + GMP	1

Enzyme	Gene	Reaction	E. coli genome
Xanthosine phosphorylase	xapA	DIN + PI <-> HYXN + DR1P	1
Purine nucleotide phosphorylase	deoD	DIN + PI <-> HYXN + DR1P	1
Xanthosine phosphorylase	xapA	DA + PI <-> AD + DR1P	1
Purine nucleotide phosphorylase	deoD	DA + PI <-> AD + DR1P	1
Xanthosine phosphorylase	xapA	DG + PI <-> GN + DR1P	1
Purine nucleotide phosphorylase	deoD	DG + PI <-> GN + DR1P	1
Xanthosine phosphorylase	xapA	HYXN + R1P <-> INS + PI	1
Purine nucleotide phosphorylase	deoD	HYXN + R1P <-> INS + PI	1
Xanthosine phosphorylase	xapA	AD + R1P <-> PI + ADN	1
Purine nucleotide phosphorylase	deoD	AD + R1P <-> PI + ADN	1
Xanthosine phosphorylase	xapA	GN + R1P <-> PI + GSN	1
Purine nucleotide phosphorylase	deoD	GN + R1P <-> PI + GSN	1
Xanthosine phosphorylase	xapA	XAN + R1P <-> PI + XTSN	1
Purine nucleotide phosphorylase	deoD	XAN + R1P <-> PI + XTSN	1
Uridine phosphorylase	udp	URI + PI <-> URA + R1P	1
Thymidine (deoxyuridine) phosphorylase	deoA	DU + PI <-> URA + DR1P	1
Purine nucleotide phosphorylase	deoD	DU + PI <-> URA + DR1P	1
Thymidine (deoxyuridine) phosphorylase	deoA	DT + PI <-> THY + DR1P	1
Cytidylate kinase	cmkA	DCMP + ATP <-> ADP + DCDP	1
Cytidylate kinase	cmkA	CMP + ATP <-> ADP + CDP	1
Cytidylate kinase	cmkB	DCMP + ATP <-> ADP + DCDP	1

Enzyme	Gene	Reaction	E. coli genome
Cytidylate kinase	cmkB	CMP + ATP <·> ADP + CDP	1
Cytidylate kinase	cmkA	UMP + ATP <·> ADP + UDP	1
Cytidylate kinase	cmkB	UMP + ATP <·> ADP + UDP	1
dTMP kinase	tmk	DTMP + ATP <·> ADP + DTDP	1
Uridylate kinase	pyrH	UMP + ATP <·> UDP + ADP	1
Uridylate kinase	pyrH	DUMP + ATP <·> DUDP + ADP	1
Thymidine (deoxyuridine) kinase	tdk	DU + ATP ·> DUMP + ADP	1
Uracil phosphoribosyltransferase	upp	URA + PRPP ·> UMP + PPI	1
Cytosine deaminase	codA	CYTS ·> URA + NH3	1
Uridine kinase	udk	URI + GTP ·> GDP + UMP	1
Cytidine kinase	udk	CYTD + GTP ·> GDP + CMP	1
CMP glycosylase		CMP ·> CYTS + R5P	0
Cytidine deaminase	cdd	CYTD ·> URI + NH3	1
Thymidine (deoxyuridine) kinase	tdk	DT + ATP ·> ADP + DTMP	1
dCTP deaminase	dcd	DCTP ·> DUTP + NH3	1
Cytidine deaminase	cdd	DC ·> NH3 + DU	1
5'-Nucleotidase	ushA	DUMP ·> DU + PI	1
5'-Nucleotidase	ushA	DTMP ·> DT + PI	1
5'-Nucleotidase	ushA	DAMP ·> DA + PI	1
5'-Nucleotidase	ushA	DGMP ·> DG + PI	1
5'-Nucleotidase	ushA	DCMP ·> DC + PI	1

Enzyme	Gene	Reaction	E. coli genome
5'-Nucleotidase	ushA	CMP -> CYTD + PI	1
5'-Nucleotidase	ushA	AMP -> PI + ADN	1
5'-Nucleotidase	ushA	GMP -> PI + GSN	1
5'-Nucleotidase	ushA	IMP -> PI + INS	1
5'-Nucleotidase	ushA	XMP -> PI + XTSN	1
5'-Nucleotidase	ushA	UMP -> PI + URI	1
Ribonucleoside-diphosphate reductase	nrdAB	ADP + RTHIO -> DADP + OTHIO	2
Ribonucleoside-diphosphate reductase	nrdAB	GDP + RTHIO -> DGDP + OTHIO	2
Ribonucleoside-triphosphate reductase	nrdD	ATP + RTHIO -> DATP + OTHIO	1
Ribonucleoside-triphosphate reductase	nrdD	GTP + RTHIO -> DGTP + OTHIO	1
Ribonucleoside-diphosphate reductase	nrdAB	CDP + RTHIO -> DCDP + OTHIO	2
Ribonucleoside-diphosphate reductase II	nrdEF	CDP + RTHIO -> DCDP + OTHIO	2
Ribonucleoside-diphosphate reductase	nrdAB	UDP + RTHIO -> DUDP + OTHIO	2
Ribonucleoside-triphosphate reductase	nrdD	CTP + RTHIO -> DCTP + OTHIO	1
Ribonucleoside-triphosphate reductase	nrdD	UTP + RTHIO -> OTHIO + DUTP	1
dUTP pyrophosphatase	dut	DUTP -> PPI + DUMP	1
Thymidilate synthetase	thyA	DUMP + METTHF -> DHF + DTMP	1
Nucleoside triphosphatase	mutT	GTP -> GSN + 3 PI	1
Nucleoside triphosphatase	mutT	DGTP -> DG + 3 PI	1
Deoxyguanosinetriphosphate triphophohydrolase	dgt	DGTP -> DG + 3 PI	1
Deoxyguanosinetriphosphate triphophohydrolase	dgt	GTP -> GSN + 3 PI	1

Enzyme	Gene	Reaction	E. coli genome
			4
Glycine cleavage system (Multi-component system)	gcvHTP, lpdA	GLY + THF + NAD -> METTHF + NADH + CO2 + NH3	
Formyl tetrahydrofolate deformylase	purU	FTHF -> FOR + THF	1
Methylene tetrahydrofolate reductase	metF	METTHF + NADH -> NAD + MTHF	1
Methylene THF dehydrogenase	folD	METTHF + NADP <-> METHF + NADPH	1
Methenyl tetrahydrofolate cyclohydrolase	folD	METHF <-> FTHF	1
Acetyl-CoA carboxyltransferase	accABD	ACCOA + ATP + CO2 <-> MALCOA + ADP + PI	3
Malonyl-CoA-ACP transacylase	fabD	MALCOA + ACP <-> MALACP + COA	1
Malonyl-ACP decarboxylase	fadB	MALACP -> ACACP + CO2	1
Acetyl-CoA-ACP transacylase	fabH	ACACP + COA <-> ACCOA + ACP	1
Acyltransferase	pls	GL3P +0.035 C140ACP +0.102 C141ACP +0.717 C160ACP +0.142 C161ACP +1.004 C181ACP -> 2 ACP + PA	0
CDP-Diacylglycerol synthetase	cdsA	PA + CTP <-> CDPDG + PPI	1
CDP-Diacylglycerol pyrophosphatase	cdh	CDPDG -> CMP + PA	1
Phosphatidylserine synthase	pssA	CDPDG + SER <-> CMP + PS	1
Phosphatidylserine decarboxylase	psd	PS -> PE + CO2	1
Phosphatidylglycerol phosphate synthase	pgsA	CDPDG + GL3P <-> CMP + PGP	1
Phosphatidylglycerol phosphate phosphatase A	pgpA	PGP -> PI + PG	0
Phosphatidylglycerol phosphate phosphatase B	pgpB	PGP -> PI + PG	1
Cardiolipin synthase	cls	2 PG <-> CL + GL	1
Acetyl-CoA C-acetyltransferase	atoB	2 ACCOA <-> COA + AACCOA	1
Isoprenyl-pyrophosphate synthesis pathway		T3P1 + PYR + 2 NADPH + ATP -> IPPP + ADP + 2 NADP + CO2	0

-33-

Enzyme	Gene	Reaction	E. coli genome
Isoprenyl pyrophosphate isomerase		IPPP -> DMPP	0
Farnesyl pyrophosphate synthetase	ispA	DMPP + IPPP -> GPP + PPI	1
Geranyltransferase	ispA	GPP + IPPP -> FPP + PPI	1
Octoprenyl pyrophosphate synthase (5 reactions)	ispB	5 IPPP + FPP -> OPP + 5 PPI	1
Undecaprenyl pyrophosphate synthase (8 reactions)		8 IPPP + FPP -> UDPP + 8 PPI	0
Chorismate pyruvate-lyase	ubiC	CHOR -> 4HBZ + PYR	1
Hydroxybenzoate octaprenyltransferase	ubiA	4HBZ + OPP -> O4HBZ + PPI	1
Octaprenyl-hydroxybenzoate decarboxylase	ubiD, ubiX	O4HBZ -> CO2 + 2OPPP	1
2-Octaprenylphenol hydroxylase	ubiB	2OPPP + O2 -> 2O6H	1
Methylation reaction		2O6H + SAM -> 2OPMP + SAH	0
2-Octaprenyl-6-methoxyphenol hydroxylase	ubiH	2OPMP + O2 -> 2OPMB	1
2-Octaprenyl-6-methoxy-1,4-benzoquinone methylase	ubiE	2OPMB + SAM -> 2OPMMB + SAH	0
2-Octaprenyl-3-methyl-6-methoxy-1,4-benzoquinone hydroxylase	ubiF	2OPMMB + O2 -> 2OMHMB	0
3-Dimethylubiquinone 3-methyltransferase	ubiG	2OMHMB + SAM -> OH2 + SAH	1
Isochorismate synthase 1	menF	CHOR -> ICHOR	1
α-Ketoglutarate decarboxylase	menD	AKG + TPP -> SSALTPP + CO2	1
SHCHC synthase	menD	ICHOR + SSALTPP -> PYR + TPP + SHCHC	1
O-Succinylbenzoate-CoA synthase	menC	SHCHC -> OSB	1
O-Succinylbenzoic acid-CoA ligase	menE	OSB + ATP + COA -> OSBCOA + AMP + PPI	1
Naphthoate synthase	menB	OSBCOA -> DHNA + COA	1

Enzyme	Gene	Reaction	E. coli genome
1,4-Dihydroxy-2-naphthoate octaprenyltransferase	menA	DHNA + OPP -> DMK + PPI + CO2	1
S-Adenosylmethionine-2-DMK methyltransferase	menG	DMK + SAM -> MK + SAH	1
Isochorismate synthase 2	entC	CHOR -> ICHOR	1
Isochorismatase	entB	ICHOR <-> 23DHDHB + PYR	1
2,3-Dihydo-2,3-dihydroxybenzoate dehydrogenase	entA	23DHDHB + NAD <-> 23DHB + NADH	1
ATP-dependent activation of 2,3-dihydroxybenzoate	entE	23DHB + ATP <-> 23DHBA + PPI	1
ATP-dependent serine activating enzyme	entF	SER + ATP <-> SERA + PPI	1
Enterochelin synthetase	entD	3 SERA + 3 23DHBA -> ENTER + 6 AMP	1
GTP cyclohydrolase II	ribA	GTP -> D6RP5P + FOR + PPI	1
Pyrimidine deaminase	ribD	D6RP5P -> A6RP5P + NH3	1
Pyrimidine reductase	ribD	A6RP5P + NADPH -> A6RP5P2 + NADP	1
Pyrimidine phosphatase		A6RP5P2 -> A6RP + PI	0
3,4 Dihydroxy-2-butanone-4-phosphate synthase	ribB	RL5P -> DB4P + FOR	1
6,7-Dimethyl-8-ribityllumazine synthase	ribE	DB4P + A6RP -> D8RL + PI	1
Riboflavin synthase	ribH	2 D8RL -> RIBFLV + A6RP	1
Riboflavin kinase	ribF	RIBFLV + ATP -> FMN + ADP	1
FAD synthetase	ribF	FMN + ATP -> FAD + PPI	1
GTP cyclohydrolase I	folE	GTP -> FOR + AHTD	1
Dihydroneopterin triphosphate pyrophosphorylase	ntpA	AHTD -> PPI + DHPP	1
Nucleoside triphosphatase	mutT	AHTD -> DHP + 3 PI	1
Dihydroneopterin monophosphate dephosphorylase		DHPP -> DHP + PI	0

-35-

Enzyme	Gene	Reaction	E. coli genome
Dihydroneopterin aldolase	folB	DHP -> AHHMP + GLAL	1
6-Hydroxymethyl-7,8 dihydropterin pyrophosphokinase	folK	AHHMP + ATP -> AMP + AHHMD	1
Aminodeoxychorismate synthase	pabAB	CHOR + GLN -> ADCHOR + GLU	2
Aminodeoxychorismate lyase	pabC	ADCHOR -> PYR + PABA	1
Dihydropteroate synthase	folP	PABA + AHHMD -> PPI + DHPT	1
Dihydrofolate synthetase	folC	DHPT + ATP + GLU -> ADP + PI + DHF	1
Dihydrofolate reductase	folA	DHF + NADPH -> NADP + THF	1
Ketopentoate hydroxymethyl transferase	panB	OIVAL + METTHF -> AKP + THF	1
Ketopantoate reductase	panE	AKP + NADPH -> NADP + PANT	0
Acetohyoxyacid isomeroreductase	ilvC	AKP + NADPH -> NADP + PANT	1
Aspartate decarboxylase	panD	ASP -> CO2 + bALA	1
Pantoate-β-alanine ligase	panC	PANT + bALA + ATP -> AMP + PPI + PNTO	1
Pantothenate kinase	coaA	PNTO + ATP -> ADP + 4PPNTO	1
Phosphopantothenate-cysteine ligase		4PPNTO + CTP + CYS -> CMP + PPI + 4PPNCYS	0
Phosphopantothenate-cysteine decarboxylase		4PPNCYS -> CO2 + 4PPNTE	0
Phospho-pantethiene adenylyltransferase		4PPNTE + ATP -> PPI + DPCOA	0
DephosphoCoA kinase		DPCOA + ATP -> ADP + COA	0
ACP Synthase	acpS	COA -> PAP + ACP	1
Aspartate oxidase	nadB	ASP + FAD -> FADH + ISUCC	1
Quinolate synthase	nadA	ISUCC + T3P2 -> PI + QA	1

Enzyme	Gene	Reaction	E. coli genome
Quinolate phosphoribosyl transferase	nadC	QA + PRPP -> NAMN + CO2 + PPI	1
NAMN adenylyl transferase	nadD	NAMN + ATP -> PPI + NAAD	0
NAMN adenylyl transferase	nadD	NMN + ATP -> NAD + PPI	0
Deamido-NAD ammonia ligase	nadE	NAAD + ATP + NH3 -> NAD + AMP + PPI	1
NAD kinase	nadFG	NAD + ATP -> NADP + ADP	0
NADP phosphatase		NADP -> NAD + PI	0
DNA ligase	lig	NAD -> NMN + AMP	1
NMN amidohydrolase	pncC	NMN -> NAMN + NH3	0
NMN glycohydrolase (cytoplasmic)		NMN -> R5P + NAm	0
NAm amidohydrolase	pncA	NAm -> NAC + NH3	0
NAPRTase	pncB	NAC + PRPP + ATP -> NAMN + PPI + PI + ADP	1
NAD pyrophosphatase	pnuE	NADxt -> NMNxt + AMPxt	0
NMN permease	pnuC	NMNxt -> NMN	1
NMN glycohydrolase (membrane bound)		NMNxt -> R5P + NAm	0
Nicotinic acid uptake		NACxt -> NAC	0
GSA synthetase	hemM	GLU + ATP -> GTRNA + AMP + PPI	1
Glutamyl-tRNA synthetase	gltX	GLU + ATP -> GTRNA + AMP + PPI	1
Glutamyl-tRNA reductase	hemA	GTRNA + NADPH -> GSA + NADP	1
Glutamate-1-semialdehyde aminotransferase	hemL	GSA -> ALAV	1
Porphobilinogen synthase	hemB	8 ALAV -> 4 PBG	1
Hydroxymethylbilane synthase	hemC	4 PBG -> HMB + 4 NH3	1

Enzyme	Gene	Reaction	E. coli genome
Uroporphyrinogen III synthase	hemD	HMB -> UPRG	1
Uroporphyrin-III C-methyltransferase 1	hemX	SAM + UPRG -> SAH + PC2	1
Uroporphyrin-III C-methyltransferase 2	cysG	SAM + UPRG -> SAH + PC2	1
1,3-Dimethyluroporphyrinogen III dehydrogenase	cysG	PC2 + NAD -> NADH + SHCL	1
Siroheme ferrochelatase	cysG	SHCL -> SHEME	1
Uroporphyrinogen decarboxylase	hemE	UPRG -> 4 CO2 + CPP	1
Coproporphyrinogen oxidase, aerobic	hemF	O2 + CPP -> 2 CO2 + PPHG	2
Protoporphyrinogen oxidase	hemG	O2 + PPHG -> PPIX	2
Ferrochelatase	hemH	PPIX -> PTH	1
Heme O synthase	cyoE	PTH + FPP -> HO + PPI	1
8-Amino-7-oxononanoate synthase	bioF	ALA + CHCOA <-> CO2 + COA + AONA	1
Adenosylmethionine-8-amino-7-oxononanoate aminotransferase	bioA	SAM + AONA <-> SAMOB + DANNA	1
Dethiobiotin synthase	bioD	CO2 + DANNA + ATP <-> DTB + PI + ADP	1
Biotin synthase	bioB	DTB + CYS <-> BT	1
Glutamate-cysteine ligase	gshA	CYS + GLU + ATP -> GC + PI + ADP	1
Glutathione synthase	gshB	GLY + GC + ATP -> RGT + PI + ADP	1
Glutathione reductase	gor	NADPH + OGT <-> NADP + RGT	1
thiC protein	thiC	AIR -> AHM	1
HMP kinase	thiN	AHM + ATP -> AHMP + ADP	0
HMP-phosphate kinase	thiD	AHMP + ATP -> AHMPP + ADP	0

Enzyme	Gene	Reaction	E. coli genome
Hypothetical		T3P1 + PYR -> DTP	0
thiG protein	thiG	DTP + TYR + CYS -> THZ + HBA + CO2	1
thiE protein	thiE	DTP + TYR + CYS -> THZ + HBA + CO2	1
thiF protein	thiF	DTP + TYR + CYS -> THZ + HBA + CO2	1
thiH protein	thiH	DTP + TYR + CYS -> THZ + HBA + CO2	1
THZ kinase	thiM	THZ + ATP -> THZP + ADP	0
Thiamin phosphate synthase	thiB	TH2P + AHMPP -> THMP + PPI	0
Thiamin kinase	thiK	THMP + ADP <-> THIAMIN + ATP	0
Thiamin phosphate kinase	thiL	THMP + ATP <-> TPP + ADP	0
Erythrose 4-phosphate dehydrogenase	epd	E4P + NAD <-> ER4P + NADH	1
Erythronate-4-phosphate dehydrogenase	pdxB	ER4P + NAD <-> OHB + NADH	1
Hypothetical transaminase/phosphoserine transaminase	serC	OHB + GLU <-> PHT + AKG	1
Pyridoxal-phosphate biosynthetic proteins pdxJ-pdxA	pdxAJ	PHT + DX5P -> P5P + CO2	2
Pyridoxine 5'-phosphate oxidase	pdxH	P5P + O2 <-> PL5P + H2O2	1
Threonine synthase	thrC	PHT -> 4HLT + PI	1
Hypothetical Enzyme		4HLT -> PYRDX	0
Pyridoxine kinase	pdxX	PYRDX + ATP -> P5P + ADP	1
Hypothetical Enzyme		P5P -> PYRDX + PI	0
Hypothetical Enzyme		PL5P -> PL + PI	0
Pyridoxine kinase	pdxX	PL + ATP -> PL5P + ADP	1

Enzyme	Gene	Reaction	E. coli genome
Pyridoxine 5'-phosphate oxidase	pdxH	PYRDX + O2 <-> PL + H2O2	1
Pyridoxine 5'-phosphate oxidase	pdxH	PL + O2 + NH3 <-> PDLA + H2O2	1
Pyridoxine kinase	pdxK	PDLA + ATP -> PDLA5P + ADP	1
Hypothetical Enzyme		PDLA5P -> PDLA + PI	0
Pyridoxine 5'-phosphate oxidase	pdxH	PDLA5P + O2 -> PL5P + H2O2 + NH3	1
Serine hydroxymethyltransferase (serine methylase)	glyA	PL5P + GLU -> PDLA5P + AKG	1
Serine hydroxymethyltransferase (serine methylase)	glyA	PL5P + ALA -> PDLA5P + PYR	1
Glutamine fructose-6 phosphate Transaminase	glmS	F6P + GLN -> GLU + GA6P	1
Phosphoglucosamine mutase	glmM	GA6P <-> GA1P	0
N-Acetylglucosamine-1-phosphate-uridyltransferase	glmU	UTP + GA1P + ACCOA -> UDPNAG + PPI + COA	1
UDP-N-acetylglucosamine acyltransferase	lpxA	C140ACP + UDPNAG -> ACP + UDPG2AA	1
UDP-3-O-acyl-N-acetylglucosamine deacetylase	lpxC	UDPG2AA -> UDPG2A + AC	1
UDP-3-O-(3-hydroxymyristoyl)glucosamine acyltransferase	lpxD	UDPG2A + C140ACP -> ACP + UDPG23A	1
UDP-sugar hydrolase	ushA	UDPG23A -> UMP + LIPX	1
Lipid A disaccharide synthase	lpxB	LIPX + UDPG23A -> UDP + DISAC1P	1
Tetraacyldisaccharide 4' kinase		DISAC1P + ATP -> ADP + LIPIV	0
3-Deoxy-D-manno-octulosonic-acid transferase (KDO transferase)	kdtA	LIPIV + CMPKDO -> KDOLIPIV + CMP	1
3-Deoxy-D-manno-octulosonic-acid transferase (KDO transferase)	kdtA	KDOLIPIV + CMPKDO -> K2LIPIV + CMP	1
Endotoxin synthase	htrB, msbB	K2LIPIV + C140ACP + C120ACP -> LIPA + 2 ACP	2

Enzyme	Gene	Reaction	E. coli genome
3-Deoxy-D-manno-octulosonic-acid 8-phosphate synthase	kdsA	PEP + A5P -> KDOP + PI	1
3-Deoxy-D-manno-octulosonic-acid 8-phosphate phosphatase		KDOP -> KDO + PI	0
CMP-2-keto-3-deoxyoctonate synthesis	kdsB	KDO + CTP -> PPI + CMPKDO	1
ADP-L-glycero-D-mannoheptose-6-epimerase	lpcA, rfaED	S7P + ATP -> ADPHEP + PPI	1
UDP glucose-1-phosphate uridylyltransferase	galU, galF	G1P + UTP -> PPI + UDPG	2
Ethanolamine phosphotransferase		PE + CMP <-> CDPETN + DGR	0
Phosphatidate phosphatase		PA -> PI + DGR	0
Diacylglycerol kinase	dgkA	DGR + ATP -> ADP + PA	1
LPS Synthesis – truncated version of LPS (ref neid)	rfaLIJGFC	LIPA +3 ADPHEP +2 UDPG +2 CDPETN + 3 CMPKDO -> LPS +3 ADP +2 UDP +3 CMP +2 CDP	6
UDP-N-acetylglucosamine-enolpyruvate transferase	murA	UDPNAG + PEP -> UDPNAGEP + PI	1
UDP-N-acetylglucosamine-enolpyruvate dehydrogenase	murB	UDPNAGEP + NADPH -> UDPNAM + NADP	1
UDP-N-acetylmuramate-alanine ligase	murC	UDPNAM + ALA + ATP -> ADP + PI + UDPNAMA	1
UDP-N-acetylmuramoylalanine-D-glutamate ligase	murD	UDPNAMA + DGLU + ATP -> UDPNAMAG + ADP + PI	1
UDP-N-acetylmuramoylalanyl-D-glutamate 2,6-diaminopimelate ligase	murE	UDPNAMAG + ATP + MDAP -> UNAGD + ADP + PI	1
D-Alanine-D-alanine adding enzyme	murF	UNAGD + ATP + AA -> UNAGDA + ADP + PI	1
Glutamate racemase	murI	GLU <-> DGLU	1
D-ala:D-ala ligases	ddlAB	2 DALA <-> AA	2

Enzyme	Gene	Reaction	E. coli genome
Phospho-N-acetylmuramoylpentapeptide transferase	mraY	UNAGDA -> UMP + PI + UNPTDO	1
N-Acetylglucosaminyl transferase	murG	UNPTDO + UDPNAG -> UDP + PEPTIDO	1
Arabinose (low affinity)	araE	ARABxt + HEXT <-> ARAB	1
Arabinose (high affinity)	araFGH	ARABxt + ATP -> ARAB + ADP + PI	3
Dihydroxyacetone		DHAxt + PEP -> T3P2 + PYR	0
Fructose	fruABF	FRUxt + PEP -> F1P + PYR	2
Fucose	fucP	FUCxt + HEXT <-> FUC	1
Galacitol	gatABC	GLTLxt + PEP -> GLTL1P + PYR	3
Galactose (low affinity)	galP	GLACxt + HEXT -> GLAC	1
Galactose (low affinity)	galP	GLCxt + HEXT -> GLC	1
Galactose (high affinity)	mglABC	GLACxt + ATP -> GLAC + ADP + PI	3
Glucitol	srlA1A2B	GLTxt + PEP -> GLT6P + PYR	3
Gluconate	gntST	GLCNxt + ATP -> GLCN + ADP + PI	1
Glucose	ptsG, crr	GLCxt + PEP -> G6P + PYR	2
Glycerol	glpF	GLxt <-> GL	1
Lactose	lacY	LCTSxt + HEXT <-> LCTS	1
Maltose	malX, crr, malEFGK, lamB	MLTxt + PEP -> MLT6P + PYR	7
Mannitol	mtlA, cmtAB	MNTxt + PEP -> MNT6P + PYR	3
Mannose	manXYZ, ptsPAl	MANxt + PEP -> MAN1P + PYR	6
Melibiose	melB	MELIxt + HEXT -> MELI	1

Enzyme	Gene	Reaction	E. coli genome
N-Acetylglucosamine	nagE, ptsN	NAG + PEP -> NAGP + PYR	2
Rhamnose	rha T	RMNxt + ATP -> RMN + ADP + PI	1
Ribose	rbsABCD, xylH	RIBxt + ATP -> RIB + ADP + PI	5
Sucrose	scr	SUCxt + PEP -> SUC6P + PYR	0
Trehalose	treAB	TRExt + PEP -> TRE6P + PYR	2
Xylose (low affinity)	xylE	XYLxt + HEXT -> XYL	1
Xylose (high affinity)	xylFG, rbsB	XYLxt + ATP -> XYL + ADP + PI	3
Alanine	cycA	ALAxt + ATP -> ALA + ADP + PI	1
Arginine	artPMQJI, argT hisMQP	ARGxt + ATP -> ARG + ADP + PI	9
Asparagine (low Affinity)	not identified	ASNxt + HEXT <-> ASN	0
Asparagine (high Affinity)		ASNxt + ATP -> ASN + ADP + PI	0
Aspartate	gltP	ASPxt + HEXT -> ASP	1
Aspartate	gltJKL	ASPxt + ATP -> ASP + ADP + PI	3
Branched chain amino acid transport	brnQ	BCAAxt + HEXT <-> BCAA	1
Cysteine	not identified	CYSxt + ATP -> CYS + ADP + PI	0
D-Alanine	cycA	DALAxt + ATP -> DALA + ADP + PI	1
D-Alanine glycine permease	cycA	DALAxt + HEXT <-> DALA	1
D-Alanine glycine permease	cycA	DSERxt + HEXT <-> DSER	1
D-Alanine glycine permease	cycA	GLYxt + HEXT <-> GLY	1
Diaminopimelic acid		MDAPxt + ATP -> MDAP + ADP + PI	0
γ-Aminobutyrate transport	gabP	GABAxt + ATP -> GABA + ADP + PI	1

Enzyme	Gene	Reaction	E. coli genome
Glutamate	gltP	GLUxt + HEXT <-> GLU	1
Glutamate	gltS	GLUxt + HEXT <-> GLU	1
Glutamate	gltJKL	GLUxt + ATP -> GLU + ADP + PI	3
Glutamine	glnHPQ	GLNxt + ATP -> GLN + ADP + PI	3
Glycine	cycA, proVWX	GLYxt + ATP -> GLY + ADP + PI	4
Histidine	hisJMPQ	HISxt + ATP -> HIS + ADP + PI	4
Isoleucine	livJ	ILExt + ATP -> ILE + ADP + PI	1
Leucine	livHKM/livFGJ	LEUxt + ATP -> LEU + ADP + PI	6
Lysine	lysP	LYSxt + HEXT <-> LYS	1
Lysine	argT, hisMPQ	LYSxt + ATP -> LYS + ADP + PI	4
Lysine/Cadaverine	cadB	LYSxt + ATP -> LYS + ADP + PI	1
Methionine	metD	METxt + ATP -> MET + ADP + PI	0
Ornithine	argT, hisMPQ	ORNxt + ATP -> ORN + ADP + PI	4
Phenylalanine	aroP/mtr/pheP	PHExt + HEXT <-> PHE	3
Proline	putP, proPWX	PROxt + HEXT <-> PRO	4
Proline	cycA, proVW	PROxt + ATP -> PRO + ADP + PI	4
Putrescine	potEFHIG	PTRCxt + ATP -> PTRC + ADP + PI	5
Serine	sdaC	SERxt + HEXT <-> SER	1
Serine	cycA	SERxt + ATP -> SER + ADP + PI	1
Spermidine & putrescine	potABCD	SPMDxt + ATP -> SPMD + ADP + PI	4
Spermidine & putrescine	potABCD	PTRCxt + ATP -> PTRC + ADP + PI	4

Enzyme	Gene	Reaction	E. coli genome
Threonine	livJ	THRxt + ATP -> THR + ADP + PI	1
Threonine	tdcC	THRxt + HEXT <-> THR	1
Tryptophan	tnaB	TRPxt + HEXT <-> TRP	1
Tyrosine	tyrP	TYRxt + HEXT <-> TYR	1
Valine	livJ	VALxt + ATP -> VAL + ADP + PI	1
Dipeptide	dppABCDF	DIPEPxt + ATP -> DIPEP + ADP + PI	5
Oligopeptide	oppABCDF	OPEPxt + ATP -> OPEP + ADP + PI	5
Peptide	sapABD	PEPTxt + ATP -> PEPT + ADP + PI	3
Uracil	uraA	URAxt + HEXT -> URA	1
Nicotinamide mononucleotide transporter	pnuC	NMNxt + HEXT -> + NMN	1
Cytosine	codB	CYTSxt + HEXT -> CYTS	1
Adenine	purB	ADxt + HEXT -> AD	1
Guanine	gpt, hpt	GNxt <-> GN	2
Hypoxanthine	gpt, hpt	HYXNxt <-> HYXN	2
Xanthosine	xapB	XTSNxt <-> XTSN	1
Xanthine	gpt	XANxt <-> XAN	1
G-system	nupG	ADNxt + HEXT -> ADN	1
G-system	nupG	GSNxt + HEXT -> GSN	1
G-system	nupG	URIxt + HEXT -> URI	1
G-system	nupG	CYTDxt + HEXT -> CYTD	1
G-system (transports all nucleosides)	nupG	INSxt + HEXT -> INS	1

Enzyme	Gene	Reaction	E. coli genome
G-system	nupG	XTSNxt + HEXT -> XTSN	1
G-system	nupG	DTxt + HEXT -> DT	1
G-system	nupG	DINxt + HEXT -> DIN	1
G-system	nupG	DGxt + HEXT -> DG	1
G-system	nupG	DAxt + HEXT -> DA	1
G-system	nupG	DCxt + HEXT -> DC	1
G-system	nupG	DUxt + HEXT -> DU	1
C-system	nupC	ADNxt + HEXT -> ADN	1
C-system	nupC	URIxt + HEXT -> URI	1
C-system	nupC	CYTDxt + HEXT -> CYTD	1
C-system	nupC	DTxt + HEXT -> DT	1
C-system	nupC	DAxt + HEXT -> DA	1
C-system	nupC	DCxt + HEXT -> DC	1
C-system	nupC	DUxt + HEXT -> DU	1
Nucleosides and deoxynucleoside	tsx	ADNxt + HEXT -> ADN	1
Nucleosides and deoxynucleoside	tsx	GSNxt + HEXT -> GSN	1
Nucleosides and deoxynucleoside	tsx	URIxt + HEXT -> URI	1
Nucleosides and deoxynucleoside	tsx	CYTDxt + HEXT -> CYTD	1
Nucleosides and deoxynucleoside	tsx	INSxt + HEXT -> INS	1
Nucleosides and deoxynucleoside	tsx	XTSNxt + HEXT -> XTSN	1
Nucleosides and deoxynucleoside	tsx	DTxt + HEXT -> DT	1

Enzyme	Gene	Reaction	E. coli genome
Nucleosides and deoxynucleoside	tsx	DINxt + HEXT -> DIN	1
Nucleosides and deoxynucleoside	tsx	DGxt + HEXT -> DG	1
Nucleosides and deoxynucleoside	tsx	DAxt + HEXT -> DA	1
Nucleosides and deoxynucleoside	tsx	DCxt + HEXT -> DC	1
Nucleosides and deoxynucleoside	tsx	DUxt + HEXT -> DU	1
Acetate transport		ACxt + HEXT <-> AC	0
Lactate transport		LACxt + HEXT <-> LAC	0
L-Lactate	lldP	LLACxt + HEXT <-> LLAC	1
Formate transport	focA	FORxt <-> FOR	1
Ethanol transport		ETHxt + HEXT <-> ETH	0
Succinate transport	dcuAB	SUCCxt + HEXT <-> SUCC	2
Pyruvate transport		PYRxt + HEXT <-> PYR	0
Ammonia transport	amtB	NH3xt + HEXT <-> NH3	1
Potassium transport	kdpABC	Kxt + ATP -> K + ADP + PI	3
Potassium transport	trkAEHG	Kxt + HEXT <-> K	3
Sulfate transport	cysPTUWAZ, sbp	SLFxt + ATP -> SLF + ADP + PI	7
Phosphate transport	pstABCS	PIxt + ATP -> ADP + 2 PI	4
Phosphate transport	pitAB	PIxt + HEXT <-> PI	2
Glycerol-3-phosphate	glpT, ugpABCE	GL3Pxt + PI -> GL3P	5
Dicarboxylates	dcuAB, dctA	SUCCxt + HEXT <-> SUCC	3
Dicarboxylates	dcuAB, dctA	FUMxt + HEXT <-> FUM	3

Enzyme	Gene	Reaction	E. coli genome
Dicarboxylates	dcuAB, dctA	MALxt + HEXT <-> MAL	3
Dicarboxylates	dcuAB, dctA	ASPxt + HEXT <-> ASP	3
Fatty acid transport	fadL	C140xt -> C140	1
Fatty acid transport	fadL	C160xt -> C160	1
Fatty acid transport	fadL	C180xt -> C180	1
α-Ketogluterate	kgtP	AKGxt + HEXT <-> AKG	1
Na/H antiporter	nhaABC	NAxt + <-> NA + HEXT	2
Na/H antiporter	chaABC	NAxt + <-> NA + HEXT	3
Pantothenate	panF	PNTOxt + HEXT <-> PNTO	1
Sialic acid permease	nanT	SLAxt + ATP -> SLA + ADP + PI	1
Oxygen transport		O2xt <-> O2	0
Carbon dioxide transport		CO2xt <-> CO2	0
Urea transport		UREAxt + 2 HEXT <-> UREA	0
ATP drain flux for constant maintanence requirements		ATP -> ADP + PI	0
Glyceraldehyde transport	gutP	GLALxt <-> GLAL	0
Acetaldehyde transport		ACALxt <-> ACAL	0

Table 2

Comparison of the predicted mutant growth characteristics from the gene deletion study to published experimental results with single and double mutants.

Gene	Glucose (in vivo/in silico)	Glycerol (in vivo/in silico)	Succinate (in vivo/in silico)	Acetate (in vivo/in silico)
aceEF	-/+			
aceA				-/-
aceB				-/-
ackA				+/+
acs				+/+
acn	-/-	-/-	-/-	-/-
cyd	+/+			
cyo	+/+			
eno	-/+	-/+	-/-	-/-
fba	-/+			
fbp	+/+	-/-	-/-	-/-
gap	-/-	-/-	-/-	-/-
gltA	-/-	-/-	-/-	-/-
gnd	+/+			
idh	-/-	-/-	-/-	-/-
ndh	+/+	+/+		
nuo	+/+	+/+		
pfk	-/+			
pgi	+/+	+/+		
pgk	-/-	-/-	-/-	-/-
pgl	+/+			
pntAB	+/+	+/+	+/+	+/+
glk	+/+			
ppc	±/+	-/+	+/+	+/+
pta				+/+
pts	+/+			
pyk	+/+			
rpi	-/-	-/-	-/-	-/-
sdhABCD	+/+			
tpi	-/+	-/-	-/-	-/-

Gene	Glucose (in vivo|in silico)	Glycerol (in vivo|in silico)	Succinate (in vivo|in silico)	Acetate (in vivo|in silico)
unc	+|+		-|-	-|-
zwf	+|+			
sucAD	+|+			
zwf, pnt	+|+			
pck, mez			-|-	-|-
pck, pps			-|-	-|-
pgi, zwf	-|-			
pgi, gnd	-|-			
pta,acs				-|-
tktA, tktB	-|-			

Results are scored as + or - meaning growth or no growth determined from *in vivo | in silico* data. In 73 of 80 cases the *in silico* behavior is the same as the experimentally observed behavior.

WHAT IS CLAIMED IS:

1.   A method for determining the genome specific stoichiometric matrix of an organism, comprising:

providing the nucleotide sequence of a metabolic gene in the organism;

identifying the open reading frame of the metabolic gene;

assigning a function to the metabolic gene based on its nucleotide or amino acid homology to other,

known metabolic genes;

determining the metabolic genotype of the organism based on the assigned function of the

metabolic gene; and

determining the genome specific stoichiometric matrix for the organism.

2.   The method of Claim 1, further comprising determining a phenotype of the organism.

3.   The method of Claim 2, wherein determining the phenotype of the organism comprises analyzing

the consequences of reduction or addition to the composition of the metabolic genotype.

4.   The method of Claim 2, further comprising identifying lethal genetic deletions.

5.   The method of Claim 4 further comprising determining the effectiveness of a drug through analysis

of the lethal genetic deletions.

6.   The method of Claim 1, further comprising determining the minimal media composition required to

sustain growth of the organism.

7.   The method of Claim 1, further comprising determining an optimal media composition for growing

the organism.

8.   The method of Claim 1, further comprising determining the most advantageous complement of

genes in the organism necessary to sustain growth in a particular environmental condition.

9.   The method of Claim 1, wherein the organism is *Escherichia Coli*.

10.   The method of Claim 1, comprising the use of a Flux Based Analysis on the stoichiometric matrix.

11.   The method of Claim 1, comprising adding biochemical information for a metabolic gene to the

stoichiometric matrix.

12.   A method for determining a potential genetic target for a drug that kills an organism, comprising:

providing the nucleotide sequence of a metabolic gene in the organism;

identifying the open reading frame of the metabolic gene;

assigning a function to the metabolic gene based on its nucleotide or amino acid homology to other,

known metabolic genes;

determining whether the metabolic gene is required for growth of the organism;

repeating the providing, identifying, assigning and determining steps for other metabolic genes of

the organism; and

selecting a gene that is required for growth of the organism as a target for the drug.

13.   The method of Claim 12, wherein the organism is *Escherichia coli*.

14. The method of Claim 12, comprising performing a Flux Based Analysis of a stoichiometric matrix from the organism.

15. The method of Claim 12, comprising the use of biochemical information on the metabolic gene to determine whether it is required for growth of the organism.

5   16. A computer system comprising a memory having instructions that when executed perform the steps of:

providing the nucleotide sequence of a metabolic gene in an organism;

identifying the open reading frame of the metabolic gene;

assigning a function to the metabolic gene based on its nucleotide or amino acid homology to other,

10   known metabolic genes;

determining the metabolic genotype of the organism based on the assigned function of the unknown metabolic gene; and

determining the genome specific stoichiometric matrix for the organism.

17. The computer system of Claim 16, wherein said memory is selected from the group consisting of: a

15   hard disk, optical memory, Random Access Memory, Read Only Memory and Flash Memory.

18. The computer system of Claim 16, wherein said computer system is based on an Intel® microprocessor.

19. The computer system of Claim 16, wherein the organism is *Escherichia Coli*.

20. The computer system of Claim 16, further comprising instructions that when executed perform the

20   method of identifying lethal genetic deletions for the organism.

21. The computer system of Claim 16, comprising instructions, that when executed, add biochemical information on a metabolic gene to the stoichiometric matrix.

22. A method for representing a living organism in a computer system, comprising:

providing the nucleotide sequence of a metabolic gene in the organism;

25   identifying the open reading frame of the metabolic gene;

assigning a function to the metabolic gene based on its nucleotide or amino acid homology to other, known metabolic genes;

determining the metabolic genotype of the organism based on the assigned function of the metabolic gene;

30   determining the genome specific stoichiometric matrix for the organism; and

storing the genome specific stoichiometric matrix in a memory of the computer.

23. The method of Claim 22, wherein the organism is *Escherichia coli*.

24. The method of Claim 22, comprising the use of Flux Based Analysis to analyze the stoichiometric matrix.

35   25. The method of Claim 22, comprising adding biochemical information on a metabolic gene to determine the metabolic genotype of the organism.

26.   The method of Claim 22, comprising calculating the genome specific stoichiometric matrix using Flux Based Analysis.

27.   A genome specific stoichiometric matrix representing the metabolism of a living organism, produced by a process comprising:
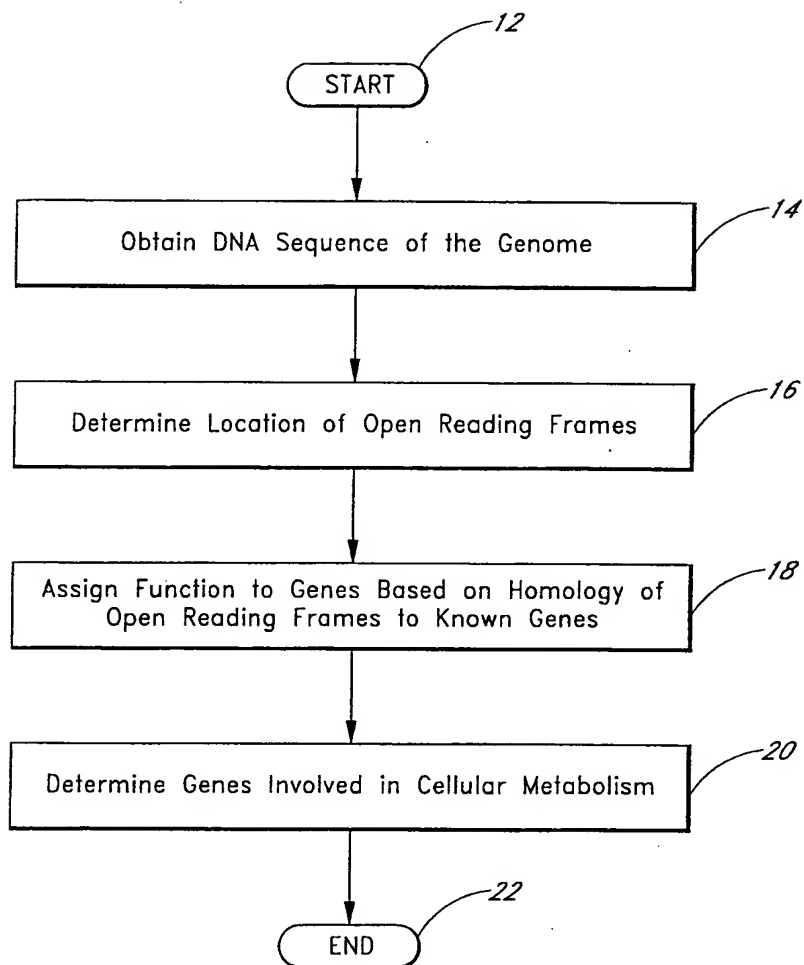
5   providing the nucleotide sequence of a metabolic gene in the organism;

identifying the open reading frame of the metabolic gene;

assigning a function to the metabolic gene based on its nucleotide or amino acid homology to other, known metabolic genes;

determining the metabolic genotype of the organism based on the assigned function of the

10   metabolic gene; and

determining the genome specific stoichiometric matrix for the organism.
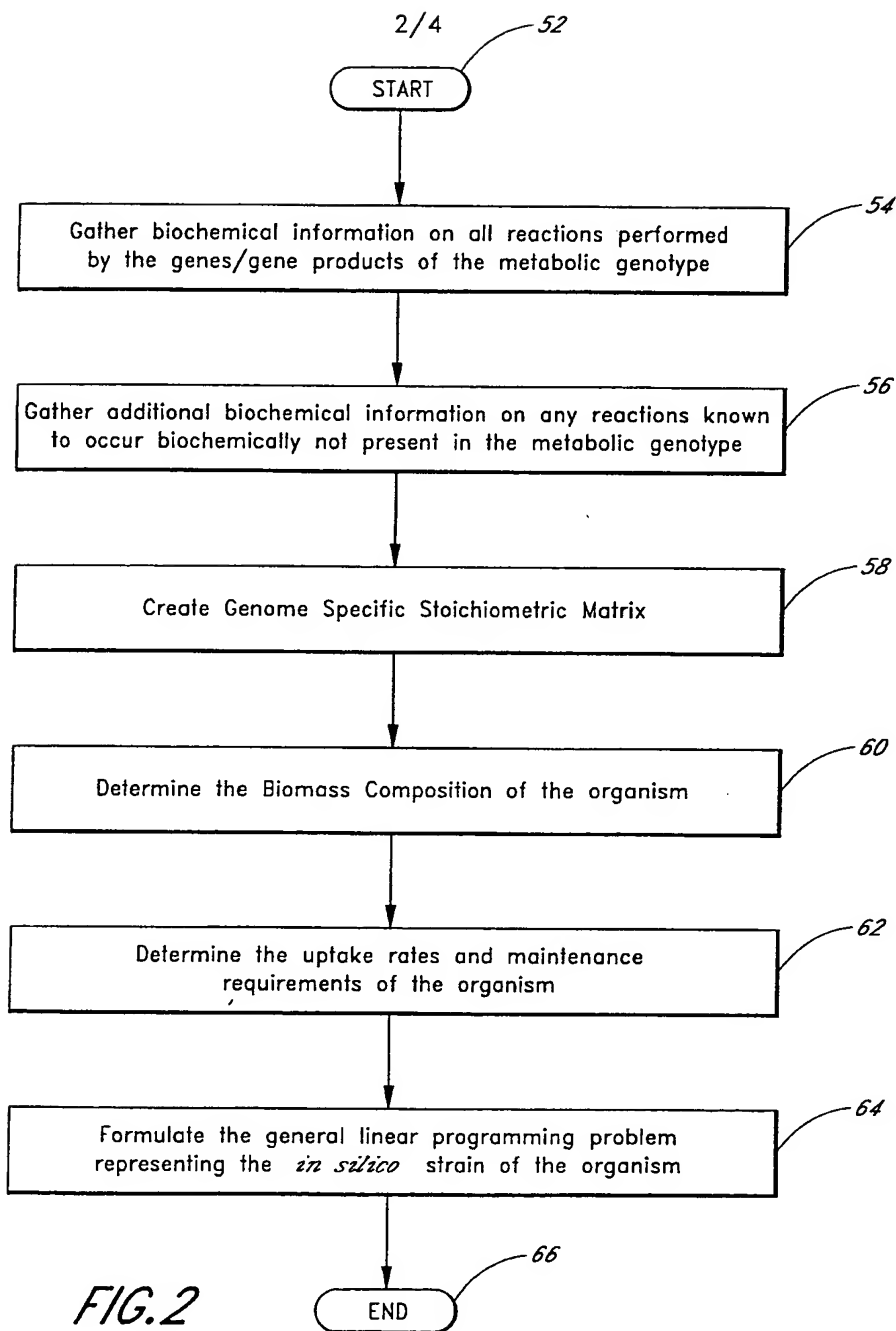
28.   The stoichiometric matrix of Claim 1, wherein the organism is *Escherichia Coli*.

29.   The stoichiometric matrix of Claim 1, wherein the stoichiometric matrix is determined using Flux Based Analysis.

15   30.   The stoichiometric matrix of Claim 1, produced by the process of adding biochemical information for the metabolic gene.

1/4

FIG. 1

2/4   *52*

START

Gather biochemical information on all reactions performed
by the genes/gene products of the metabolic genotype   *54*

Gather additional biochemical information on any reactions known
to occur biochemically not present in the metabolic genotype   *56*

Create Genome Specific Stoichiometric Matrix   *58*

Determine the Biomass Composition of the organism   *60*

Determine the uptake rates and maintenance
requirements of the organism   *62*

Formulate the general linear programming problem
representing the *in silico* strain of the organism   *64*

66

FIG.2   END

FIG.3A

4/4

Relative flux decrease Aerobic to anaerobic shift	
Gene	Change
pdh	Repression
pgl	Repression
zwf	Repression
gnd	Repression
sucAB,lpd	Repression
sdhABCD	Repression
gcvHTP	Repression
fdnGHI	Repression
trxB	Repression
sucCD	18.19
tal	14.42
fumAB	12.21
mdh	12.21
can	11.66
gltA	11.66
idh	11.66
cyoABCD	9.46
atpABCDEFGH	9.39
nuoABDEFGH IJKLMN	8.68
rpi	7.18
tktA	6.79
rpe	6.79

FIG.3B

Relative flux increase Aerobic to anaerobic shift	
Gene	Change
pfl	Induction
pyk	Induction
adhE	Induction
putT	Induction
frdABCD	Induction
ackA	16.81
pta	16.81

FIG.3C

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7   C12Q1/68   G06F19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7   G06F   C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EDWARDS JEREMY S ET AL: "How will bioinformatics influence metabolic engineering?" BIOTECHNOLOGY AND BIOENGINEERING, vol. 58, no. 2-3, 20 April 1998 (1998-04-20), pages 162-169, XP002153942 ISSN: 0006-3592 the whole document ---	1-30
X	TOMITA MASARU ET AL: "E-CELL: Software environment for whole-cell simulation." BIOINFORMATICS (OXFORD), vol. 15, no. 1, January 1999 (1999-01), pages 72-84, XP000961128 ISSN: 1367-4803 the whole document --- -/--	1-30

[X] Further documents are listed in the continuation of box C.   [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not
      considered to be of particular relevance
"E" earlier document but published on or after the international
      filing date
"L" document which may throw doubts on priority claim(s) or
      which is cited to establish the publication date of another
      citation or other special reason (as specified)
"O" document referring to an oral disclosure. use, exhibition or
      other means
"P" document published prior to the international filing date but
      later than the priority date claimed

"T" later document published after the international filing date
      or priority date and not in conflict with the application but
      cited to understand the principle or theory underlying the
      invention
"X" document of particular relevance; the claimed invention
      cannot be considered novel or cannot be considered to
      involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention
      cannot be considered to involve an inventive step when the
      document is combined with one or more other such docu-
      ments, such combination being obvious to a person skilled
      in the art.
"&" document member of the same patent family

Date of the actual completion of the international search

27 November 2000

Date of mailing of the international search report

12/12/2000

Name and mailing address of the ISA
            European Patent Office, P.B. 5818 Patentlaan 2
            NL – 2280 HV Rijswijk
            Tel. (+31–70) 340–2040, Tx. 31 651 epo nl,
            Fax: (+31–70) 340–3016

Authorized officer

Reuter, U

Form PCT/ISA/210 (second sheet) (July 1992)

page 1 of 4

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	BONARIUS H P J ET AL: "Flux analysis of underdetermined metabolic networks: the quest for the missing constraints" TRENDS IN BIOTECHNOLOGY,GB,ELSEVIER PUBLICATIONS, CAMBRIDGE, vol. 15, no. 8, 1 August 1997 (1997-08-01), pages 308-314, XP004083358 ISSN: 0167-7799 the whole document	1-30
X	PALSSON: "What lies beyond bioinformatics?" NATURE BIOTECHNOLOGY, vol. 15, January 1997 (1997-01), pages 3-4, XP002153943 page 4	1-30
X	SCHILLING CHRISTOPHE H ET AL: "The underlying pathway structure of biochemical reaction networks." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 95, no. 8, 14 April 1998 (1998-04-14), pages 4193-4198, XP002153944 April 14, 1998 ISSN: 0027-8424 the whole document	1-30
X	ARIGONI ET AL: "A GENOME-BASED APPROACH FOR THE IDENTIFICATION OF ESSENTIAL BACTERIAL GENES" NATURE BIOTECHNOLOGY,US,NATURE PUBLISHING, vol. 16, no. 9, September 1998 (1998-09), pages 851-856, XP002119419 ISSN: 1087-0156 the whole document	12,13
X	BALTZ ET AL: "DNA sequence sampling of the Streptococcus pneumoniae genome to identify novel targets for antibiotic development" MICROBIAL DRUG RESISTANCE,US,LIEBERT, vol. 4, no. 1, 21 March 1998 (1998-03-21), pages 1-9, XP002112153 ISSN: 1076-6294 the whole document	12,13

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	PRAMANIK J ET AL: "Stoichiometric model of Escherichia coli metabolism: Incorporation of growth-rate dependent biomass composition and mechanistic energy requirements." BIOTECHNOLOGY AND BIOENGINEERING, vol. 56, no. 4, 1997, pages 398-421, XP002153946 ISSN: 0006-3592 the whole document ---	27-30
X	SAUER UWE ET AL: "Metabolic capacity of Bacillus subtilis for the production of purine nucleosides, riboflavin, and folic acid." BIOTECHNOLOGY AND BIOENGINEERING, vol. 59, no. 2, 20 July 1998 (1998-07-20), pages 227-238, XP002153947 ISSN: 0006-3592 the whole document ---	27-30
A	KARP PETER D ET AL: "EcoCyc: Encyclopedia of Escherichia coli genes and metabolism." NUCLEIC ACIDS RESEARCH, vol. 25, no. 1, 1997, pages 43-50, XP002153948 ISSN: 0305-1048 the whole document ---	1-30
A	KARP P D: "Metabolic databases" TIBS TRENDS IN BIOCHEMICAL SCIENCES,EN,ELSEVIER PUBLICATION, CAMBRIDGE, vol. 23, no. 3, 1 March 1998 (1998-03-01), pages 114-116, XP004111317 ISSN: 0968-0004 the whole document ---	1-30
A	GALPERIN M Y ET AL: "Using metabolic pathway databases for functional annotation" TRENDS IN GENETICS,NL,ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, vol. 14, no. 8, 1 December 1998 (1998-12-01), pages 332-333, XP004131498 ISSN: 0168-9525 the whole document ---	1-30

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	XIE L ET AL: "Integrated approaches to the design of media and feeding strategies for fed-batch cultures of animal cells" TRENDS IN BIOTECHNOLOGY,GB,ELSEVIER PUBLICATIONS, CAMBRIDGE, vol. 15, no. 3, 1 March 1997 (1997-03-01), pages 109-113, XP004054783 ISSN: 0167-7799 the whole document	1,6,7
X,P	SCHILLING CHRISTOPHE H ET AL: "Toward metabolic phenomics: Analysis of genomic data using flux balances." BIOTECHNOLOGY PROGRESS, vol. 15, no. 3, May 1999 (1999-05), pages 288-295, XP000961124 ISSN: 8756-7938 the whole document	1-30